IVD-1033-S

# chemagic™
# Viral DNA/RNA 300 Kit H96

Instructions for use. Reagents for 960 extractions.

Manufacturer:

PerkinElmer® chemagen Technologie GmbH (BAE),
Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany
www.chemagen.com, www.perkinelmer.com
Phone: +49 (0) 2401805500

CE

**FOR *IN VITRO* DIAGNOSTIC USE. VERSION 220914 EN**

## SYMBOLS

| Symbol | Symbol Title |
|---|---|
| CE | Symbol CE |
| LOT | Batch code |
| REF | Catalog number |
|  | Use-by date |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Manufacturer |
|  | GHS02 |
|  | GHS05 |

| Symbol | Symbol Title |
|---|---|
|  | GHS07 |
|  | GHS08 |
|  | Dangerous goods: Class 3 Flammable liquid |
|  | Dangerous goods: Class 8 Corrosive substances |
|  | This way up |
|  | Recyclable |
|  | Fragile, handle with care |
|  | Keep dry |

## chemagic™ Viral DNA/RNA 300 Kit H96

### APPLICATION

The chemagic™ Viral DNA/RNA 300 Kit H96 is to be used for the automated extraction and purification of DNA and RNA from human plasma, saliva and naso- or oropharyngeal swabs using the chemagic™ 360-D instrument (prod. no. 2024-0010).

The kit is designed to be used with IVD downstream applications employing enzymatic amplification and detection of DNA and RNA (e.g. PCR, RT-PCR, NGS). The product is intended for trained laboratory personnel. For further information please refer to the "REAGENTS" section and the "WARNINGS AND PRECAUTIONS" section in this document.

### SUMMARY AND PRINCIPLE

The chemagic™ Viral DNA/RNA 300 Kit H96 is based on a magnetic bead technology platform proprietary to PerkinElmer® chemagen Technologie GmbH. Cells or other source of DNA/RNA present in plasma, serum and naso- or oropharyngeal swabs are lysed during the extraction process. The released nucleic acids bind to small magnetizable particles which are then magnetically separated from the sample material. During subsequent steps contaminants are removed and the purified nucleic acids are transferred into an elution buffer. The automated sample processing is performed using the chemagic™ 360-D instrument (prod. no. 2024-0010) with a chemagic™ 96 Rod Head Set (prod. no. CMG-370) or equivalent instrument.

To minimize irregularities in diagnostic results, the product is intended to be used with an internal control as well as positive and negative controls throughout the process of sample preparation, sample amplification and detection according to the downstream assay used.

For a patient/user/third party in the European Union and in countries with an identical regulatory regime (IVDR (EU) 2017/746); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to your national authority and to the manufacturer and PerkinElmer® chemagen Technologie GmbH, +49 (0) 2401805500 or support.chemagen@perkinelmer.com or it´s legal representatives.

### KIT CONTENTS

The kit contains reagents sufficient to perform 960 extractions.

The expiry date of the unopened kit is stated on the outer label. Do not use any component beyond the expiry date. Store at +2 to +25 °C.

Once opened, the kit components have a limited stability. The stability after opening is stated for each component separately in the reagent listing below. Note: Recap the bottles tightly

immediately after use to prevent evaporation.

The bottles may discolor during storage. The discoloration of the bottles has no effect on the functionality of the assay.

## ELECTRONIC INSTRUCTIONS FOR USE

Electronic Instructions for Use (eIFU) in different languages are available on our webpage. To download these electronic Instructions for Use please visit

https://chemagen.com/ivd-1033-s-chemagic-viral-dna-rna-300-kit-h96/.

The eIFU are provided in English (EN), French (FR), Spanish (ES) and Italian (IT).

In case of any questions regarding download or the electronic Instructions for Use please contact us: support.chemagen@perkinelmer.com or +49 (0) 2401805500.

## PROTOCOL FILES

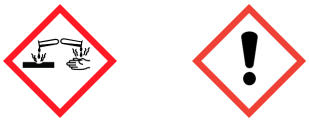
Kit-related protocol files are available on the webpage or will be provided by customer support (see above).

## REAGENTS

IVD-1033-S - chemagic™ Viral DNA/RNA 300 Kit H96

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Magnetic Beads** | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Suspension of particles containing nanoparticular iron oxide encapsulated in a matrix of polyvinyl alcohol. Magnetic Beads bind the DNA/RNA during the extraction process.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Lysis Buffer 1**<br>  <br>DANGER | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Store in the dark.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Ready-for-use aqueous buffer solution containing guanidine thiocyanate (50–70 %). Lysis Buffer is used to lyse the cells or other DNA/RNA source present in the sample in order to get the DNA/RNA in solution.

**LYSIS BUFFER 1 CONTAINS GUANIDINIUM THIOCYANATE:**

H302+H312 Harmful if swallowed or in contact with skin.
H314 Causes severe skin burns and eye damage.
P101 If medical advice is needed, have product container or label at hand.
P102 Keep out of reach of children.
P103 Read carefully and follow all instructions.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER/doctor.
P321 Specific treatment (see on this label).
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

EUH032 Contact with acids liberates very toxic gas.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Binding Buffer 2**<br>  <br>DANGER | 1 canister<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Ready-for-use Tris-HCl-buffered (pH 5.2–6.1) solution with sodium perchlorate (20–40 %) and ethanol (40–60 %). Binding Buffer 2 is used to create the appropriate conditions to get the DNA/RNA bound to the Magnetic Beads.

**BINDING BUFFER 2 CONTAINS ETHANOL AND SODIUM PERCHLORATE:**

H225 Highly flammable liquid and vapor.
H302 Harmful if swallowed.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P240 Ground and bond container and receiving equipment.
P241 Use explosion-proof [electrical/ventilating/lighting] equipment.
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Wash Buffer 3**<br>  <br>DANGER | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Ready-for-use Tris-HCl-buffered (pH 4.8-5.6) solution with sodium perchlorate (20–30 %) and ethanol (20–40 %). Used for removing non-DNA/non-RNA contaminants during washing step.

**WASH BUFFER 3 CONTAINS ETHANOL AND SODIUM PERCHLORATE:**

H225 Highly flammable liquid and vapor.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P240 Ground and bond container and receiving equipment.
P241 Use explosion-proof [electrical/ventilating/lighting] equipment.
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Wash Buffer 4**<br>  <br>DANGER | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Ready-for-use solution contains ethanol 50–70 %. Used for removing last traces of non-DNA/non-RNA contaminants during washing step.

**WASH BUFFER 4 CONTAINS ETHANOL:**

H225 Highly flammable liquid and vapor.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P240 Ground and bond container and receiving equipment
P241 Use explosion-proof [electrical/ventilating/lighting] equipment.
P280 Wear protective gloves/protective clothing/eye protection/face protection
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Wash Buffer 5** | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Ready-for-use ultra-filtered water solution. Used for removing possible residuals of ethanol.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Elution Buffer 6** | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once opened, stable for 60 days at +2 to +25 °C. |

Ready-for-use 10 mM Tris-HCl-buffered (pH 7.8–8.4) solution.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Proteinase K**<br>  <br>DANGER | 1 bottle (lyophilized) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once reconstituted,<br>stable for 28 days at +2 to +8 °C. |

The Proteinase K is reconstituted by adding 11 mL of purified water. Proteinase K is added to enhance the efficiency of the lysis step.

**PROTEINASE K CONTAINS PROTEINASE, TRITIRACHIUM ALBUM SERINE AND CALCIUM ACETATE HYDRATE:**

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust/fume/gas/mist/vapors/spray.
P280 Wear protective gloves/eye protection/face protection.
P284 [In case of inadequate ventilation] wear respiratory protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Poly(A)RNA** | 10 tubes (dried) | +2 to +25 °C until expiry date stated on the bottle label.<br>Once reconstituted,<br>stable for 30 days at +2 to +8 °C. |

Poly(A)RNA is reconstituted by adding 440 µL of Poly(A)RNA Buffer. The Poly(A)RNA functions as a DNA/RNA carrier to enhance the efficiency of the extraction process.

| Component | Quantity | Shelf life and storage |
|---|---|---|
| **Poly(A)RNA Buffer**<br>  <br>DANGER | 1 bottle<br>(volume see label) | +2 to +25 °C until expiry date stated on the bottle label. |

Ready-for-use aqueous buffer solution containing guanidine thiocyanate (20–40 %). Poly(A)RNA Buffer is used for reconstitution of Poly(A)RNA.

**POLY(A)RNA BUFFER CONTAINS GUANIDINIUM THIOCYANATE:**

H302 Harmful if swallowed.
P264 Wash thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P330 Rinse mouth.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.
EUH032 Contact with acids liberates very toxic gas.

| Component | Quantity | Storage |
|---|---|---|
| **Disposable Tips** (96 ea) | 10 x 96 ea | +2 to +25 °C |
| **Deep Well Plates** (5 ea) | 10 x 5 ea | +2 to +25 °C |
| **Low Well Plates** (5 ea) | 2 x 5 ea | +2 to +25 °C |

## MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The chemagic™ Viral DNA/RNA 300 Kit H96 requires the following items, which are available from PerkinElmer®, Inc. and its distributors:

- chemagic™ 360-D instrument (prod. no. 2024-0010) with chemagic™ 96 Rod Head Set (prod. no. CMG-370)

Additional required items:

- Kit-related protocol file (.che-file) provided by PerkinElmer® chemagen Technologie GmbH (see section “PROTOCOL FILES”, p. 4)
- pipettes and pipette tips with aerosol barriers
- molecular grade water

Additional optional items:

- chemagic™ Stand 96 (prod. no. CMG-301)
- isotonic saline solution, sterile
- Sarstedt tube (cat. no. 72.693 or 72.694)

## SPECIMEN COLLECTION AND HANDLING

The chemagic™ Viral DNA/RNA 300 Kit H96 is usable with fresh and frozen human plasma, stabilized with either EDTA or citrate from common blood collection systems, stabilized saliva (Oragene™ and Spectrum™ collection tubes) and transport media from swabs (e.g. eNAT™ Copan Diagonstics Inc.) as direct aliquots of 300 µL per isolation.

After collection and centrifugation, plasma can be stored at 2–8 °C for up to 6 hours. For long-term storage, freezing at –20 °C or –80 °C in aliquots is recommended. Frozen plasma or serum samples must not be thawed more than once. Repeated freezing–thawing leads to denaturation and precipitation of proteins, resulting in reduced yields of nucleic acids.

Sample material from dried swabs must be transferred into isotonic saline solution. Therefore add 350 µL of isotonic saline solution and incubate for 5 min at 15-25 °C before use. 300 µL of the incubated isotonic saline solution sample should be used per isolation.

**NOTE: DO NOT USE PHOSPHATE CONTAINING BUFFERS FOR RESUSPENSION.**

The extraction efficiency of sample material other than the sample types listed above has not been determined.

For safe handling specimen for viral testing (e.g. SARS-CoV-2 viral RNA extraction) should be inactivated before use. Pipette 4 µL Poly(A)RNA, 10 µL Proteinase K, and 300 µL Lysis Buffer 1 into a 2 mL Sarstedt tube (cat. no. 72.693 or 72.694). Note: When more than one sample will be processed for inactivation, a stock solution of this solution can be prepared. Simply multiply the volumes required for one sample by the total number of samples to be processed and include additional volume to the equivalent of 3 extra samples. Invert the tube several times to mix, transfer 314 µL to a 2 mL Sarstedt tube for each sample, and then continue for each sample by adding 300 µL sample to each tube, close the lid, and mix by pulse-vortexing for 10 seconds. Incubate the tube at 68 °C for 15 minutes (± 2 minutes) for inactivation. Transfer the inactivated lysate completely to the sample deep well plate in step 3 of the extraction protocol and continue with step 4.

## WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

The product is intended for professional users trained for the chemagic™ 360-D instrument (prod. no. 2024-0010).

Handle all specimens as potentially infectious. Potentially infectious samples shall be inactivated. Please refer to the U.S. Department of Health and Human Services publication "Biosafety in Microbiological and Biomedical Laboratories" or any other local or national regulation.

Lysis Buffer 1 contains guanidinium thiocyanate and is harmful if swallowed, in contact with skin or if inhaled. Binding Buffer 2 and Wash Buffer 3 contain sodium perchlorate and ethanol and are flammable liquids and vapors and are harmful if swallowed. Wash Buffer 4 contains ethanol and is a flammable liquid and vapor. Proteinase K contains Tritirachium album serine Proteinase and causes skin irritation and serious eye irritation, may cause allergy or asthma symptoms or breathing difficulties if inhaled and respiratory irritation. Poly(A)RNA Buffer contains guanidinium thiocyanate and is harmful if swallowed or if inhaled. See specific precautions for all components in the section “REAGENTS”.

To avoid injuries when working with the kit components, always wear safety glasses, disposable gloves, and protective clothing. For detailed information, please refer to the corresponding safety data sheets (SDS).

Follow local regulations for handling of ethanolic solutions.

Disposal of all waste should be in accordance with local regulations.

## PROCEDURE 60 MIN PROTOCOL (VARIOUS SPECIES)

Extraction protocol using the chemagic™ 360-D instrument.

The duration of the automated extraction protocol is approximately 60 minutes.

The protocol is suitable for processing up to 96 samples in parallel (see “PROCESSING STEPS IN DETAIL” below). For detailed instructions on the use of the chemagic™ 360-D instrument, please refer to the chemagic™ 360-D User Manual.

Samples and reagents must be brought to room temperature (+19 to +25 °C) before use. Connect the reagent bottles to the chemagic™ 360-D instrument as follows:

| | |
|---|---|
| Pump 1: | No bottle connected |
| Pump 2: | Binding Buffer 2 |
| Pump 3: | Wash Buffer 3 |
| Pump 4: | Wash Buffer 4 |
| Pump 5: | Wash Buffer 5 |
| Pump 6: | No bottle connected |

**NOTE: RECAP THE BOTTLES TIGHTLY IMMEDIATELY AFTER USE OR KEEP THE BOTTLES CONNECTED TIGHTLY TO THE CHEMAGIC™ 360-D INSTRUMENT. BINDING BUFFER 2, WASH BUFFER 3 AND WASH BUFFER 4 CONTAIN ETHANOL. IF ETHANOL EVAPORATES, THE OPTIMAL YIELD OR DETECTION SENSITIVITY CANNOT BE GUARANTEED.**

### PROCESSING STEPS IN DETAIL

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. Before prefilling the plates mark each plate with material in position (samples, Magnetic Beads and buffers).
3. Reconstitute the Proteinase K and Poly(A)RNA components.
    - Proteinase K: Add 11 mL molecular biology grade water to Proteinase K bottle and mix gently until dissolved.
    - Poly(A)RNA: Add 440 µL of Poly(A)RNA Buffer to the Poly(A)RNA tube and mix thoroughly until dissolved.
4. If Lysis Buffer 1 contains precipitate (formed during transfer or storage), the solution should be heated to 50–60 °C and thoroughly mixed until the solution is clear. The clarity of the Lysis Buffer 1 should always be visually confirmed before use.
5. Fill and prime the chemagic™ 360-D tubing with reagents by choosing the protocol “**prime manifolds H96 all 360 V150116.che**”. Press [Insert IDs], follow the instructions given in the chemagic™ QA software and start priming by pressing [OK]. If functions

enabling the ID data input are deactivated, start priming directly by pressing [Start]. Priming needs to be done when reagent bottles are connected to the chemagic™ 360-D instrument for the first time or when the instrument's tubing is not already filled with the above mentioned reagents.

6. If priming is not needed, select the protocol "**check manifolds 1 – 6 H96 all 360 V150116.che**" and press [Insert IDs] or - if the enhanced functions are deactivated - [Start]. A small volume of buffer will be dispensed by each pump sequentially starting with the first pump used for this application. If one of the pumps does not show dispensing of buffer through all nozzles, please use the corresponding priming protocol for this pump. Performing several runs a day it is only necessary to check the pumps once at the beginning of the day.
7. Select the protocol "**chemagic Viral300 360 H96 prefilling VD200617.che**" and press [Insert IDs] and follow the instructions given in the chemagic™ QA software.
8. Use Disposable Tips according to the positions of the samples and place the Tip Tray in position 1 on the tracking system.
9. Check the volumes in the buffer supply containers and confirm by pressing [OK].

**NOTE: TAKE CARE THAT ALL BUFFER SUPPLY BOTTLES CONTAIN ENOUGH BUFFER. ONLY IF THE LIQUID LEVEL FOR ALL BUFFERS IS ABOVE 125 mL 96 ISOLATIONS CAN BE PERFORMED.**

10. Select the number of samples for prefilling by using the drop down menu. The scheme for positioning the samples will be shown after selecting. Take care to use the given positions. Confirm by pressing [OK].
11. Prefill the selected wells of the sample plate with 300 µL sample. To ensure the homogeneity of the samples, mix the samples gently prior to pipetting in the wells of the sample plate.

**NOTE: SAMPLE MATERIAL FROM DRIED SWABS HAS TO BE LIQUEFIED BEFORE USE.**

12. Prefill the Elution Buffer 6 and the thoroughly resuspended Magnetic Beads according to the sample positions:
    - Magnetic Beads (in plate position 2 on chemagic™ 360-D instrument) are resuspended by mixing thoroughly and pipetted manually (150 µL / well) to each corresponding sample well in use.

**NOTE: THE MAGNETIC BEAD SUSPENSION SHOULD BE MIXED VIGOROUSLY BEFORE DISPENSING; OTHERWISE THE SUSPENSION IS NOT HOMOGENOUS AND THE DNA/RNA YIELD COULD BE LOW.**

- Elution Buffer 6 (in plate position 7 on chemagic™ 360-D instrument) is pipetted manually (50–100 µL / well) to each corresponding sample well in use.

13. Add 4 µL Poly(A)RNA, 10 µL Proteinase K and then 300 µL Lysis Buffer 1 to the wells containing sample. It is possible to premix Poly(A)RNA, Proteinase K and Lysis Buffer 1 (choose the appropriate volume of Poly(A)RNA / Proteinase K / Lysis Buffer 1 to ensure you have sufficient volume for the number of isolations).

**NOTE: THE PROTEINASE K ACTIVITY WILL DECREASE AFTER INCUBATION LONGER THAN 10 MINUTES IN LYSIS BUFFER 1. ENSURE THAT ALL SAMPLES ARE MIXED WITH POLY(A)RNA / PROTEINASE K / LYSIS BUFFER 1 WITHIN THIS TIME.**

14. Place the plates on the tracking system according to the instructions given by the chemagic™ QA software.
15. Place the sample plate in position 3 on the tracking system.
16. Check all plates for accurate orientation and fitting.
17. Close the front door and start the process by pressing [Start]. The automated DNA/RNA extraction process is initiated.

Automated DNA/RNA extraction run on chemagic™ 360-D instrument (60 min protocol):

| Position on tracking system | Material in position | Protocol step in detail |
|---|---|---|
| | | Select the protocol "**check manifolds H96 all 360 V150116.che**" to flush the tubing prior to starting the automated extraction run. Press [Insert IDs], follow the instructions given in the chemagic™ QA software and start flushing by pressing [OK]. |
| | | When using the functions enabling the ID data input, select the protocol "**chemagic Viral300 360 H96 prefilling VD200617.che**" and press [Insert IDs]. Follow the instructions given in the chemagic™ QA software to fill in the required data.<br>Load the plates on the tracking system positions 1-7. |
| 1 | Tray with Disposable Tips | Use Disposable Tips according to the positions of the samples and place Tray with Disposable Tips.<br>**NOTE: TIPS NEED TO BE PRESENT IN TRAY IN FULL ROWS.** |
| 2 | Low well plate with 150 µL Magnetic Beads | Pipette thoroughly resuspended Magnetic Beads in each well in use according to the sample plate and place the plate. |
| 3 | Sample plate (Deep well plate) | Place the plate with prepared samples (300 µL sample, 4 µL Poly(A)RNA, 10 µL Proteinase K, and 300 µL Lysis Buffer 1).<br>Binding Buffer 2 is dispensed in the plate automatically. |
| 4 | Deep well plate | Place empty plate.<br>Wash Buffer 3 is dispensed in the plate automatically. |
| 5 | Deep well plate | Place empty plate.<br>Wash Buffer 4 is dispensed in the plate automatically. |
| 6 | Deep well plate | Place empty plate.<br>Wash Buffer 5 is dispensed in the plate automatically. |
| 7 | Deep well plate with 50–100 µL Elution Buffer 6 | Pipette (50-100 µL) Elution Buffer 6 in each well in use according to the sample positions and place the plate. |

| Position on tracking system | Material in position | Protocol step in detail |
|---|---|---|
| | | Check all plates for accurate orientation and fitting. After all plates are in place, press [OK].<br><br>Close the front door and start the DNA/RNA extraction process immediately by pressing [Start]. Subsequently the sample lysate will be mixed automatically.<br><br>If the functions enabling the ID data input are deactivated, load the plates on the tracking system positions 1-7. After all plates are in place, select the protocol "**chemagic Viral300 360 H96 prefilling VD200617.che**", mark the columns in use on the plate map in the dialog and start the extraction run directly by pressing [Start]. |

Numbers on tracking system refer to the positioning of the plate on the chemagic™ 360-D instrument.

After the isolation procedure has finished use the [Turn Table] button to unload the tracking system. Each click on [Turn Table] moves the tracking system (table) clockwise by one position.

**NOTE: NEVER MOVE THE TRACKING SYSTEM (TABLE) MANUALLY. THIS MIGHT DAMAGE THE INSTRUMENT. ALL MOVEMENTS MUST BE PERFORMED WITH THE [TURN TABLE] FUNCTION.**

**PROCEDURAL NOTES 60 MIN PROTOCOL (VARIOUS SPECIES)**

1. A thorough understanding of this IFU and the chemagic™ 360-D User Manual is necessary for successful use of the chemagic™ Viral DNA/RNA 300 Kit H96. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits with different lot numbers.
2. Do not use kit reagents after the expiry date printed on the kit label. Once opened, the reagents can be used for the time period stated in the reagent listing of this IFU.
3. Any deviation from the protocol may affect the results.
4. The reagents are automatically dispensed in whole rows and therefore the tip covers (Disposable Tips) should be used also in whole rows on each rod in contact with any reagent solution. It should also be noted that if partial plates are run, the solutions may not be sufficient for 960 extractions.
5. Opening the chemagic™ 360-D instrument door while the automated extraction run is ongoing, will terminate the run and the samples in process may be lost.
6. Cleaning and maintenance of the system is described in detail in the chemagic™ 360-D User Manual.
    a) The system cleaning is performed once per week: Clean the chemagic™ Dispenser. Select the protocol "**regular cleaning procedure 96 dispenser 360 V150116.che**" and press [Insert IDs] or [Start] if the enhanced functions are deactivated. Follow the instructions as given in the software.
    b) Prior to the next use of the chemagic™ Dispenser perform the appropriate priming protocol.
    c) The cleaning of the chemagic™ Dispenser with 70 % ethanol is recommended once per month. Simply use the "**intensive cleaning procedure H96 dispenser 360 V150116.che**" instead of the regular one for this purpose.
    d) If the chemagic™ Dispenser will not be used for longer period of time, it is mandatory to perform the "regular cleaning procedure" to maintain the performance of the instrument when bringing it back into service.

## PERFORMANCE CHARACTERISTICS

When using this extraction kit with the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay (Catalog Number: COVID-19-PCR-AUS-C) the following LoD data see below was reported (data generated by Suzhou Sym-Bio Lifescience Co., Ltd. No. 115, North Taiping Road, Taicang, Jiangsu Province, China).

## LOD USING CHEMAGIC™ 360-D INSTRUMENT FOR EXTRACTION AND APPLIED BIOSYSTEMS™ 7500 PCR SYSTEM

Samples were prepared using pooled clinical oropharyngeal swabs or nasopharyngeal swabs specimen matrix. The pooled matrix was tested using PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay and confirmed to be negative. A total of six 2-fold dilutions of known concentrations of inactivated SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) were prepared in the negative clinical matrix and processed using chemagic™ Viral DNA/RNA 300 Kit H96 (CMG-1033) on chemagic™ 360-D instrument. Six individual extraction replicates per dilution were tested. The results are summarized in the following tables.

**Table 1:** Preliminary LoD study using oropharyngeal swabs on chemagic™ 360-D instrument.

| Oropharyngeal Swab | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dilution fold | N | | ORF1ab | | Mean Ct | | |
| | Conc. (copies/mL) | Detection Rate | Conc. (copies/mL) | Detection Rate | N | ORF1ab | IC |
| 2.0E+04 | 137.00 | 6/6 | 41.85 | 6/6 | 36.48 | 36.82 | 32.18 |
| 4.0E+04 | 68.50 | 6/6 | 20.93 | 6/6 | 37.04 | 37.98 | 32.14 |
| 8.0E+04 | 34.25 | 6/6 | 10.46 | 6/6 | 39.10 | 38.88 | 32.21 |
| 1.6E+05 | 17.13 | 5/6 | 5.23 | 4/6 | 38.89 | 39.77 | 32.35 |
| 3.2E+05 | 8.56 | 3/6 | 2.62 | 2/6 | 39.35 | 39.85 | 32.28 |
| 6.4E+05 | 4.28 | 0/6 | 1.31 | 0/6 | / | / | 32.41 |
| Negative | 0 | 0/6 | 0 | 0/6 | / | / | 32.23 |

**Table 2:** Probit predicted 95% detection rate using oropharyngeal swabs spiked with SARS-CoV-2 (Isolate 2/231/human/2020/CHN) on chemagic™ 360-D instrument.

| Probit predicted 95% Detection Rate (copies/mL) | |
|---|---|
| N | ORF1ab |
| 19.08<br>(95% CI: 14.50 – 37.12) | 7.14<br>(95% CI: 5.34 – 24.00) |

**Table 3:** Preliminary LoD study using nasopharyngeal swabs on chemagic™ 360-D instrument.

| Nasopharyngeal Swab | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dilution fold | N | | ORF1ab | | Mean Ct | | |
| | Conc. (copies/mL) | Detection Rate | Conc. (copies/mL) | Detection Rate | N | ORF1ab | IC |
| 2.0E+04 | 137.00 | 6/6 | 41.85 | 6/6 | 36.65 | 36.55 | 32.32 |
| 4.0E+04 | 68.50 | 6/6 | 20.93 | 6/6 | 38.17 | 36.78 | 32.38 |
| 8.0E+04 | 34.25 | 6/6 | 10.46 | 6/6 | 38.55 | 38.24 | 32.60 |
| 1.6E+05 | 17.13 | 4/6 | 5.23 | 6/6 | 39.40 | 40.50 | 32.59 |
| 3.2E+05 | 8.56 | 2/6 | 2.62 | 1/6 | 39.59 | 40.53 | 32.86 |
| 6.4E+05 | 4.28 | 2/6 | 1.31 | 2/6 | 39.50 | 39.70 | 32.28 |
| Negative | 0 | 0/6 | 0 | 0/6 | / | / | 32.33 |

**Table 4**: Probit predicted 95% detection rate using nasopharyngeal swabs spiked with SARS- CoV-2 (Isolate 2/231/human/2020/CHN) on chemagic™ 360-D instrument.

| Probit predicted 95% Detection Rate (copies/mL) | |
|---|---|
| N | ORF1ab |
| 26.44<br>(95% CI: 18.34 – 69.51) | 8.32<br>(95% CI: 5.83 – 20.69) |

## VERIFICATION OF LOD USING CHEMAGIC™ 360-D INSTRUMENT FOR EXTRACTION AND APPLIED BIOSYSTEMS™ 7500 PCR SYSTEM

For the LoD verification study, pooled negative oropharyngeal swab matrix and pooled negative nasopharyngeal swab matrix was spiked with inactivated SARS-CoV-2 virus at the tentative LoD that was predicted among the two SARS-CoV-2 targets for each matrix (7.14 copies/mL of ORF1ab for oropharyngeal swab matrix and 8.32 copies/mL of ORF1ab for nasopharyngeal swab matrix). Twenty replicates per specimen matrix were prepared and extracted using the chemagic™ Viral DNA/RNA 300 Kit H96 (CMG-1033) on the chemagic™ 360-D instrument and tested using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay. Twenty additional replicates prepared at 1.5x the tentative LoD were also tested. The results are summarized in the following tables.

**Table 5**: chemagic™ 360-D instrument LoD verification results for oropharyngeal swab.

| Concentration (copies/mL) | | | Detection Rate | | Mean Ct | | |
|---|---|---|---|---|---|---|---|
| LoD | N | ORF1ab | N | ORF1ab | N | ORF1ab | IC |
| 1X | 23.38 | 7.14 | 95%<br>(19/20) | 95%<br>(19/20) | 38.44 | 38.76 | 33.13 |
| 1.5X | 35.07 | 10.71 | 100%<br>(20/20) | 100%<br>(20/20) | 38.74 | 38.11 | 33.09 |

**Table 6:** chemagic™ 360-D instrument LoD verification results for nasopharyngeal swab.

| Concentration (copies/mL) | | | Detection Rate | | Mean Ct | | |
|---|---|---|---|---|---|---|---|
| LoD | N | ORF1ab | N | ORF1ab | N | ORF1ab | IC |
| 1X | 27.25 | 8.32 | 95% (19/20) | 95% (19/20) | 38.53 | 38.44 | 33.81 |
| 1.5X | 40.87 | 12.49 | 100% (20/20) | 100% (20/20) | 38.50 | 37.79 | 32.72 |

## LOD VERIFICATION USING CHEMAGIC™ 360-D INSTRUMENT AND ALTERNATIVE PCR SYSTEMS (EQUIVALENCY OF PCR SYSTEMS)

To expand the use of the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay for use with the Applied Biosystems™ 7500 Fast / QuantStudio™ 3 / QuantStudio™ 5 Real- Time PCR Systems and Analytik Jena qTOWER3 / qTower3 84 Real-Time PCR system, a study was conducted using contrived clinical nasopharyngeal swab specimens. Pooled negative nasopharyngeal swab specimens were spiked with two or three known concentrations of SeraCare RNA reference material containing the entire SARS-CoV-2 viral genome (https://www.seracare.com/AccuPlex-SARSCoV2-Molecular-Controls-Kit--Full-Genome-0505-0159/). Nucleic acids were extracted using the chemagic™ Viral DNA/RNA 300 Kit H96 (CMG-1033) on the chemagic™ 360-D instrument and up to 20 individual extraction replicates were tested on each PCR instrument platforms according to the instructions for use. Testing on the original Applied Biosystems™ 7500 PCR System was included in this study for equivalency comparison. The results are summarized in the following tables. The LoD was confirmed to be 20 copies/mL for ABI7500, ABI 7500 Fast Dx, QuantStudio™ 3, QuantStudio™ 5 and qTower3 84, and 10 copies/mL for qTower3. The detection sensitivity of all six instruments is considered equivalent.

**Table 7:** LoD verification on alternate Applied Biosystems™ PCR platforms.

| Instrument | Concentration (copies/mL) | Target Gene | Mean Ct | Detection Rate for Target Gene | Overall Detection Rate for Algorithm |
|---|---|---|---|---|---|
| ABI 7500 | 6.7 | N | 40.2 | 80% (16/20) | 90% (18/20) |
| | | ORF | 39.4 | 75% (15/20) | |
| | 20 | N | 37.8 | 95% (19/20) | 100% (20/20) |
| | | ORF | 37.5 | 95% (19/20) | |
| ABI 7500 Fast Dx | 6.7 | N | 38.1 | 45% (9/20) | 90% (18/20) |
| | | ORF | 39.0 | 85% (17/20) | |
| | 20 | N | 37.7 | 75% (15/20) | 100% (20/20) |
| | | ORF | 37.5 | 100% (20/20) | |
| QS3 | 12 | N | ND | 0% (0/3) | 67% (2/3) |
| | | ORF | 34.1 | 67% (2/3) | |
| | 20 | N | 35.7 | 30% (6/20) | 100% (20/20) |
| | | ORF | 35.3 | 95% (19/20) | |
| | 60 | N | 35.8 | 45% (9/20) | 95% (19/20) |
| | | ORF | 33.0 | 95% (19/20) | |
| QS5 | 12 | N | ND | 0% (0/3) | 0% (0/3) |
| | | ORF | ND | 0% (0/3) | |
| | 20 | N | 35.8 | 25% (5/20) | 95% (19/20) |
| | | ORF | 37.0 | 95% (19/20) | |
| | 60 | N | 36.3 | 55% (11/20) | 100% (20/20) |
| | | ORF | 35.1 | 100% (20/20) | |
| qTower$^3$ | 6.7 | N | 39.3 | 30% (6/20) | 75% (15/20) |
| | | ORF | 39.7 | 65% (13/20) | |
| | 10 | N | 38.2 | 65% (13/20) | 100% (20/20) |
| | | ORF | 37.8 | 95% (19/20) | |
| | 20 | N | 38.5 | 75% (15/20) | 100% (20/20) |
| | | ORF | 36.9 | 100% (20/20) | |
| | 40 | N | 37.9 | 95% (19/20) | 100% (20/20) |
| | | ORF | 36.1 | 100% (20/20) | |
| qTower$^3$84 | 10 | N | 38.5 | 35% (7/20) | 90% (18/20) |
| | | ORF | 38.4 | 80% (16/20) | |
| | 20 | N | 39.0 | 55% (11/20) | 95% (19/20) |
| | | ORF | 37.3 | 85% (17/20) | |
| | 40 | N | 38.0 | 80% (16/20) | 100% (20/20) |
| | | ORF | 36.7 | 100% (20/20) | |

## LOD VERIFICATION IN SALIVA MATRIX BACKGROUND

The LoD (20 copies/mL) determined on QuantStudio™ 5 in the nasopharyngeal swab matrix background (described in section above) was further verified in saliva matrix background using the same instrument. Briefly, SARS-CoV-2 reference control material was spiked into negative saliva matrix to prepare positive samples at 20 copies/mL. In total 20 extraction replicates of this positive sample were extracted on chemagic™ 360-D instrument and amplified on QuantStudio™ 5. The results are summarized in the following table and LoD 20 copies/mL was verified by a 20/20 detection rate in the saliva matrix background.

**Table 8:** chemagic™ 360-D instrument LoD verification results for saliva.

| Concentration (copies/mL) | Detection Rate | | Mean Ct | | |
|---|---|---|---|---|---|
| | N | ORF1ab | N | ORF1ab | IC |
| 20 | 100% (20/20) | 100% (20/20) | 35.53 | 35.14 | 30.70 |

When using this extraction kit with the EURORealTime SARS-CoV-2 Real-time RT-PCR Assay (REF MP 2606-0110) the following LoD data see below was reported by EUROIMMUN (a PerkinElmer® company).

## LOD – ANALYTICAL SENSITIVITY ON VARIOUS PCR

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive.
First, a tentative LoD was determined by testing 5-7 serial dilutions prepared by spiking recombinant virus containing SARS-CoV-2 RNA (Seracare, AccuPlex™ SARS-CoV-2 Reference Material; 5000 copies/mL) into oropharyngeal swab matrix negative for SARS-CoV-2. Each dilution was tested with 3 individual extraction replicates. The tentative LoD was determined to be 150 copies/mL.
The tentative LoD was confirmed by testing 21 replicates of negative oropharyngeal swab matrix spiked independently with the AccuPlex™ reference material and extracted with the CMG-1033 chemagic™ Viral DNA/RNA 300 Kit H96 on the chemagic™ 360-D instrument. Replicates were tested on the Roche LightCycler 480 II. The final LoD for all extraction methods was determined to be 150 copies/mL. The LoD of 150 copies/mL was then verified for the Applied Biosystems™ 7500 Fast Real-Time PCR, Bio-Rad CFX 96 Touch, and the Analytik Jena qTOWER$^3$ cyclers using the same procedure described above. The LoD was confirmed by testing 21 extraction replicates.

**Table 9:** LoD confirmation in oropharyngeal swab specimens.

| Instrument | Valid Replicates | SARS-CoV-2 | | IC | | SARS-CoV-2 RNA Detection Rate |
|---|---|---|---|---|---|---|
| | | n | Mean Ct | n | Mean Ct | |
| CMG-1033 chemagic™ Viral DNA/RNA 300 Kit H96 | | | | | | |
| Roche LightCycler 480 II | 21 | 20 | 37.68 | 21 | 30.39 | 95% |
| Applied Biosystems™ 7500 Fast | 21 | 21 | 36.87 | 21 | 29.97 | 100% |
| Bio-Rad CFX 96 Touch | 21 | 20 | 36.42 | 21 | 30.38 | 95% |
| Analytic Jena qTOWER$^3$ | 21 | 20 | 37.25 | 21 | 28.49 | 95% |

**PROCEDURE 31 MIN PROTOCOL (ONLY TESTED WITH SARS-COV-2 ISOLATION)**

Extraction protocol using the chemagic™ 360-D instrument.

The duration of the automated extraction protocol is approximately 31 minutes.

The protocol is suitable for processing up to 96 samples in parallel (see “PROCESSING STEPS IN DETAIL” below). For detailed instructions on the use of the chemagic™ 360-D instrument, please refer to the chemagic™ 360-D User Manual.

Samples and reagents must be brought to room temperature (+19 to +25 °C) before use. Connect the reagent bottles to chemagic™ 360-D instrument as follows:

Pump 1: No bottle connected
Pump 2: Binding Buffer 2
Pump 3: No bottle connected
Pump 4: Wash Buffer 4
Pump 5: Wash Buffer 5
Pump 6: No bottle connected

**NOTE: RECAP THE BOTTLES TIGHTLY IMMEDIATELY AFTER USE OR KEEP THE BOTTLES CONNECTED TIGHTLY TO THE CHEMAGIC™ 360-D INSTRUMENT. BINDING BUFFER 2 AND WASH BUFFER 4 CONTAIN ETHANOL. IF ETHANOL EVAPORATES, THE OPTIMAL YIELD OR DETECTION SENSITIVITY CANNOT BE GUARANTEED.**

## PROCESSING STEPS IN DETAIL

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. Before prefilling the plates mark each plate with material in position (samples, Magnetic Beads and buffers).
3. Reconstitute the Proteinase K and Poly(A)RNA components.
    - Proteinase K: Add 11 mL molecular biology grade water to Proteinase K bottle and mix gently until dissolved.
    - Poly(A)RNA: Add 440 µL of Poly(A)RNA Buffer to the Poly(A)RNA tube and mix thoroughly until dissolved.
4. If Lysis Buffer 1 contains precipitate (formed during transfer or storage), the solution should be heated to 50–60 °C and thoroughly mixed until the solution is clear. The clarity of the Lysis Buffer 1 should always be visually confirmed before use.
5. Fill and prime the chemagic™ 360-D tubing with reagents by choosing the protocol "**prime manifolds H96 all 360 V150116.che**" Press [Insert IDs], follow the instructions given in the chemagic™ QA software and start priming by pressing [OK]. If functions enabling the ID data input are deactivated, start priming directly by pressing [Start]. Priming needs to be done when reagent bottles are connected to the chemagic™ 360-D instrument for the first time or when the instrument's tubing is not already filled with the reagents.
6. If priming is not needed, select the protocol "**check manifolds 1 – 6 H96 all 360 V150116.che**" and press [Insert IDs] or – if the enhanced functions are deactivated – [Start]. A small volume of buffer will be dispensed by each pump sequentially starting with the first pump used for this application. If one of the pumps does not show dispensing of buffer through all nozzles, please use the corresponding priming protocol for this pump. Performing several runs a day it is only necessary to check the pumps once at the beginning of the day.
7. Select the protocol "**chemagic Viral300 360 H96 prefilling 31 min VD201008.che**" and press [Insert IDs], follow the instructions given in the chemagic™ QA software.
8. Use Disposable Tips according to the positions of the samples and place the Tip Tray in position 1 on the tracking system.
9. Check the volumes in the buffer supply containers and confirm by pressing [OK].

**NOTE: TAKE CARE THAT ALL BUFFER SUPPLY BOTTLES CONTAIN ENOUGH BUFFER. ONLY IF THE LIQUID LEVEL FOR ALL BUFFERS IS ABOVE 125 mL 96 ISOLATIONS CAN BE PERFORMED.**

10. Select the number of samples for prefilling by using the drop down menu. The scheme for positioning the samples will be shown after selecting. Take care to use the given positions. Confirm by pressing [OK].
11. Prefill the selected wells of the sample plate with 300 µL sample. To ensure the homogeneity of the samples, mix the samples gently prior to pipetting in the wells of the sample plate.

**NOTE: SAMPLE MATERIAL FROM DRIED SWABS HAS TO BE LIQUEFIED BEFORE USE.**

12. Prefill the Elution Buffer 6 and the thoroughly resuspended Magnetic Beads according to the sample positions:
    - Magnetic Beads (in plate position 2 on chemagic™ 360-D instrument) are resuspended by mixing thoroughly and pipetted manually (150 µL / well) to each corresponding sample well in use.

**NOTE: THE MAGNETIC BEAD SUSPENSION SHOULD BE MIXED VIGOROUSLY BEFORE DISPENSING; OTHERWISE THE SUSPENSION IS NOT HOMOGENOUS AND THE DNA/RNA YIELD COULD BE LOW.**

    - Elution Buffer 6 (in plate position 7 on chemagic™ 360-D instrument) is pipetted manually (50–100 µL / well) to each corresponding sample well in use.

13. Add 4 µL Poly(A)RNA, 10 µL Proteinase K and then 300 µL Lysis Buffer 1 to the wells containing sample. It is possible to premix Poly(A)RNA, Proteinase K and Lysis Buffer 1 (choose the appropriate volume of Poly(A)RNA / Proteinase K / Lysis Buffer 1 to ensure you have sufficient volume for the number of isolations).

**NOTE: THE PROTEINASE K ACTIVITY WILL DECREASE AFTER INCUBATION LONGER THAN 10 MINUTES IN LYSIS BUFFER 1. ENSURE THAT ALL SAMPLES ARE MIXED WITH POLY(A)RNA / PROTEINASE K / LYSIS BUFFER 1 WITHIN THIS TIME.**

14. Place the plates on the tracking system according to the instructions given by the chemagic™ QA software.
15. Place the sample plate in position 3 on the tracking system.
16. Check all plates for accurate orientation and fitting.
17. Close the front door and start the process by pressing [Start]. The automated DNA/RNA extraction process is initiated.

Automated DNA/RNA extraction run on chemagic™ 360-D instrument (31 min protocol):

| Position on tracking system | Material in position | Protocol step in detail |
|---|---|---|
| | | Select the protocol "**check manifolds H96 all 360 V150116.che**" to flush the tubing prior to starting the automated extraction run. Press [Insert IDs], follow the instructions given in the chemagic™ QA software and start flushing by pressing [OK]. |
| | | When using the functions enabling the ID data input, select the protocol "**chemagic Viral300 360 H96 prefilling 31 min VD201008.che**" and press [Insert IDs]. Follow the instructions given in the chemagic™ QA software to fill in the required data.<br>Load the plates on the tracking system positions 1-7. |
| 1 | Tray with Disposable Tips | Use Disposable Tips according to the positions of the samples and place Tray with Disposable Tips.<br>**NOTE: TIPS NEED TO BE PRESENT IN TRAY IN FULL ROWS.** |
| 2 | Low well plate with 150 µL Magnetic Beads | Pipette thoroughly resuspended Magnetic Beads in each well in use according to the sample plate and place the plate. |
| 3 | Sample plate (Deep well plate) | Place the plate with prepared samples (300 µL sample, 4 µL Poly(A)RNA, 10 µL Proteinase K, and 300 µL Lysis Buffer 1). Binding Buffer 2 is dispensed in the plate automatically. |
| 4 | Empty | - |
| 5 | Deep well plate | Place empty plate.<br>Wash Buffer 4 is dispensed to the plate automatically. |
| 6 | Deep well plate | Place empty plate.<br>Wash Buffer 5 is dispensed to the plate automatically. |
| 7 | Deep well plate with 50–100 µL Elution Buffer 6 | Pipette (50-100 µL) Elution Buffer 6 in each well in use according to the sample positions and place the plate. |

| Position on tracking system | Material in position | Protocol step in detail |
|---|---|---|
| | | Check all plates for accurate orientation and fitting. After all plates are in place, press [OK].<br><br>Close the front door and start the DNA/RNA extraction process immediately by pressing [Start]. Subsequently the sample lysate will be mixed automatically.<br><br>If the functions enabling the ID data input are deactivated, load the plates on the tracking system positions 1-7. After all plates are in place, select the protocol "**chemagic Viral300 360 H96 prefilling 31 min VD201008.che**", mark the columns in use on the plate map in the dialog and start the extraction run directly by pressing [Start]. |

Numbers on tracking system refer to the positioning of the plate on the chemagic™360-D instrument.

After the isolation procedure has finished use the [Turn Table] button to unload the tracking system. Each click on [Turn Table] moves the tracking system (table) clockwise by one position.

**NOTE: NEVER MOVE THE TRACKING SYSTEM (TABLE) MANUALLY. THIS MIGHT DAMAGE THE INSTRUMENT. ALL MOVEMENTS MUST BE PERFORMED WITH THE [TURN TABLE] FUNCTION.**

**PROCEDURAL NOTES 31 MIN PROTOCOL (ONLY TESTED WITH SARS-COV-2 ISOLATION)**

1. A thorough understanding of this package insert and the chemagic™ 360-D User Manual is necessary for successful use of the chemagic™ Viral DNA/RNA 300 Kit H96. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers.
2. Do not use kit reagents after the expiry date printed on the kit label. Once opened, the reagents can be used for the time period stated in the reagent listing of IFU.
3. Any deviation from the protocol may affect the results.
4. The reagents are automatically dispensed in whole rows and therefore the tip covers (Disposable Tips) should be used also in whole rows on each rod in contact with any reagent solution. It should also be noted that if partial plates are run, the solutions may not be sufficient for 960 extractions.
5. Opening the chemagic™ 360-D instrument door while the automated extraction run is ongoing, will terminate the run and the samples in process may be lost.
6. Cleaning and maintenance of the system is described in detail in the chemagic™360-D User Manual.
    a. The system cleaning is performed once per week: Clean the chemagic™ Dispenser. Select the protocol "**regular cleaning procedure 96 dispenser 360 V150116.che**" and press [Insert IDs] or [Start] if the enhanced functions are deactivated. Follow the instructions as given in the software.
    b. Prior to the next use of the chemagic™ Dispenser perform the appropriate priming protocol.
    c. The cleaning of the chemagic™ Dispenser with 70 % ethanol is recommended once per month. Simply use the "**intensive cleaning procedure H96 dispenser 360 V150116.che**" instead of the regular one for this purpose.
    d. If the chemagic™ Dispenser will not be used for longer period of time, it is mandatory to perform the "regular cleaning procedure" to maintain the performance of the instrument when bringing it back into service.

## PERFORMANCE NOTES 31 MIN PROTOCOL (ONLY TESTED WITH SARS-COV-2 ISOLATION)

For comparison of the 60 minutes protocol and the 31 minutes protocol extractions were performed using AccuPlex™ SARS-CoV-2 Reference Material (https://www.seracare.com/AccuPlex-SARSCoV2-Reference-Material-Kit-0505-0126/) spiked into transport medium from eNAT™ collection devices (Copan Italia S.p.A.) as sample material. The qPCR performance was tested with the EURORealTime SARS-CoV-2 qPCR (EUROIMMUN a PerkinElmer® company; kit used according to manufacturer instructions) run on a QuantStudio™ 5 Real-Time PCR System (96-well, 0.2 mL, desktop, Applied Biosystems™, A28574). The 31 minutes protocol for SARS-CoV-2 ("**chemagic Viral300 360 H96 prefilling 31 min VD201008.che**") gives the users the chance to double their daily COVID-testing capacities. This shorter protocol can be used without any modifications or calibrations on the chemagic™ 360-D instrument. There is just a Ct value shift of 0.5 – 1 Ct compared to the 60 minutes standard protocol. Thus, the sensitivity is hardly reduced although there is a huge runtime and throughput benefit.

## PROCEDURE 18 MIN PROTOCOL (ONLY TESTED WITH SARS-COV-2 ISOLATION)

Extraction protocol using the chemagic™ 360-D instrument.

The duration of the automated extraction protocol is approximately 18 minutes.

The protocol is suitable for processing up to 96 samples in parallel (see "PROCESSING STEPS IN DETAIL" below). For detailed instructions on the use of the chemagic™ 360-D instrument, please refer to the chemagic™ 360-D User Manual.

Samples and reagents must be brought to room temperature (+19 to +25 °C) before use. Connect the reagent bottles to chemagic™ 360-D instrument as follows:

| | |
|---|---|
| Pump 1: | No bottle connected |
| Pump 2: | Binding Buffer 2 |
| Pump 3: | No bottle connected |
| Pump 4: | Wash Buffer 4 |
| Pump 5: | Wash Buffer 5 |
| Pump 6: | No bottle connected |

**NOTE: RECAP THE BOTTLES TIGHTLY IMMEDIATELY AFTER USE OR KEEP THE BOTTLES CONNECTED TIGHTLY TO THE CHEMAGIC™ 360-D INSTRUMENT. BINDING BUFFER 2 AND WASH BUFFER 4 CONTAIN ETHANOL. IF ETHANOL EVAPORATES, THE OPTIMAL YIELD OR DETECTION SENSITIVITY CANNOT BE GUARANTEED.**

## PROCESSING STEPS IN DETAIL

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. Before prefilling the plates mark each plate with material in position (samples, Magnetic Beads and buffers).
3. Reconstitute the Proteinase K and Poly(A)RNA components.
    - Proteinase K: Add 11 mL molecular biology grade water to Proteinase K bottle and mix gently until dissolved.
    - Poly(A)RNA: Add 440 µL of Poly(A)RNA Buffer to the Poly(A)RNA tube and mix thoroughly until dissolved.
4. If Lysis Buffer 1 contains precipitate (formed during transfer or storage), the solution should be heated to 50–60 °C and thoroughly mixed until the solution is clear. The clarity of the Lysis Buffer 1 should always be visually confirmed before use.
5. Fill and prime the chemagic™360-D tubing with reagents by choosing the protocol "**prime manifolds H96 all 360 V150116.che**" Press [Insert IDs], follow the instructions given in the chemagic™ QA software and start priming by pressing [OK]. If functions enabling the ID data input are deactivated, start priming directly by pressing [Start]. Priming needs to be done when reagent bottles are connected to the chemagic™360-D instrument for the first time or when the instrument's tubing is not already filled with the reagents.
6. If priming is not needed, select the protocol "**check manifolds 1 – 6 H96 all 360 V150116.che**" and press [Insert IDs] or – if the enhanced functions are deactivated – [Start]. A small volume of buffer will be dispensed by each pump sequentially starting with the first pump used for this application. If one of the pumps does not show dispensing of buffer through all nozzles, please use the corresponding priming protocol for this pump. Performing several runs a day it is only necessary to check the pumps once at the beginning of the day.
7. Select the protocol "**chemagic Viral300 360 H96 prefilling 18 min VD210204.che**" and press [Insert IDs], follow the instructions given in the chemagic™ QA software.
8. Use Disposable Tips according to the positions of the samples and place the Tip Tray in position 1 on the tracking system.
9. Check the volumes in the buffer supply containers and confirm by pressing [OK].

**NOTE: TAKE CARE THAT ALL BUFFER SUPPLY BOTTLES CONTAIN ENOUGH BUFFER. ONLY IF THE LIQUID LEVEL FOR ALL BUFFERS IS ABOVE 125 mL 96 ISOLATIONS CAN BE PERFORMED.**

10. Select the number of samples for prefilling by using the drop down menu. The scheme for positioning the samples will be shown after selecting. Take care to use the given positions. Confirm by pressing [OK].
11. Prefill the selected wells of the sample plate with 300 µL sample. To ensure the homogeneity of the samples, mix the samples gently prior to pipetting them on the sample plate.

**NOTE: SAMPLE MATERIAL FROM DRIED SWABS HAS TO BE LIQUEFIED BEFORE USE.**

12. Prefill the Elution Buffer 6 and the thoroughly resuspended Magnetic Beads according to the sample positions:
    - Magnetic Beads (in plate position 2 on chemagic™ 360-D instrument) are resuspended by mixing thoroughly and pipetted manually (150 µL / well) to each corresponding sample well in use.

**NOTE: THE MAGNETIC BEAD SUSPENSION SHOULD BE MIXED VIGOROUSLY BEFORE DISPENSING; OTHERWISE THE SUSPENSION IS NOT HOMOGENOUS AND THE DNA/RNA YIELD COULD BE LOW.**

    - Elution Buffer 6 (in plate position 7 on chemagic™ 360-D instrument) is pipetted manually (50–100 µL / well) to each corresponding sample well in use.

13. Add 4 µL Poly(A)RNA, 10 µL Proteinase K and then 300 µL Lysis Buffer 1 to the wells containing sample. It is possible to premix Poly(A)RNA, Proteinase K and Lysis Buffer 1 (choose the appropriate volume of Poly(A)RNA / Proteinase K / Lysis Buffer 1 to ensure you have sufficient volume for the number of isolations).

**NOTE: THE PROTEINASE K ACTIVITY WILL DECREASE AFTER INCUBATION LONGER THAN 10 MINUTES IN LYSIS BUFFER 1. ENSURE THAT ALL SAMPLES ARE MIXED WITH POLY(A)RNA / PROTEINASE K / LYSIS BUFFER 1 WITHIN THIS TIME.**

14. Place the plates on the tracking system according to the instructions given by the chemagic™ QA software.
15. Place the sample plate in position 3 on the tracking system.
16. Check all plates for accurate orientation and fitting.
17. Close the front door and start the process by pressing [Start]. The automated DNA/RNA extraction process is initiated.

Automated DNA/RNA extraction run on chemagic™ 360-D instrument (18 min protocol):

| Position on tracking system | Material in position | Protocol step in detail |
|---|---|---|
| | | Select the protocol "**check manifolds H96 all 360 V150116.che**" to flush the tubing prior to starting the automated extraction run. Press [Insert IDs], follow the instructions given in the chemagic™ QA software and start flushing by pressing [OK]. |
| | | When using the functions enabling the ID data input, select the protocol "**chemagic Viral300 360 H96 prefilling 18 min VD210204.che**" and press [Insert IDs]. Follow the instructions given in the chemagic™ QA software to fill in the required data.<br>Load the plates on the tracking system positions 1-7. |
| 1 | Tray with Disposable Tips | Use Disposable Tips according to the positions of the samples and place Tray with Disposable Tips.<br>**NOTE: TIPS NEED TO BE PRESENT IN TRAY IN FULL ROWS.** |
| 2 | Low well plate with 150 µL Magnetic Beads | Pipette thoroughly resuspended Magnetic Beads in each well in use according to the sample plate and place the plate. |
| 3 | Sample plate (Deep well plate) | Place the plate with prepared samples (300 µL sample, 4 µL Poly(A)RNA, 10 µL Proteinase K, and 300 µL Lysis Buffer 1). Binding Buffer 2 is dispensed to the plate automatically. |
| 4 | Empty | - |
| 5 | Deep well plate | Place empty plate.<br>Wash Buffer 4 is dispensed to the plate automatically. |
| 6 | Deep well plate | Place empty plate.<br>Wash Buffer 5 is dispensed to the plate automatically. |
| 7 | Deep well plate with 50–100 µL Elution Buffer 6 | Pipette (50-100 µL) Elution Buffer 6 in each well in use according to the sample positions and place the plate. |

| Position on tracking system | Material in position | Protocol step in detail |
|---|---|---|
| | | Check all plates for accurate orientation and fitting. After all plates are in place, press [OK].<br><br>Close the front door and start the DNA/RNA extraction process immediately by pressing [Start]. Subsequently the sample lysate will be mixed automatically.<br><br>If the functions enabling the ID data input are deactivated, load the plates on the tracking system positions 1-7. After all plates are in place, select the protocol "**chemagic Viral300 360 H96 prefilling 18 min VD210204.che**", mark the columns in use on the plate map in the dialog and start the extraction run directly by pressing [Start]. |

Numbers on tracking system refer to the positioning of the plate on the chemagic™360-D instrument.

After the isolation procedure has finished use the [Turn Table] button to unload the tracking system. Each click on [Turn Table] moves the tracking system (table) clockwise by one position.

**NOTE: NEVER MOVE THE TRACKING SYSTEM (TABLE) MANUALLY. THIS MIGHT DAMAGE THE INSTRUMENT. ALL MOVEMENTS MUST BE PERFORMED WITH THE [TURN TABLE] FUNCTION.**

**PROCEDURAL NOTES 18 MIN PROTOCOL (ONLY TESTED WITH SARS-COV-2 ISOLATION)**

1. A thorough understanding of this package insert and the chemagic™ 360-D User Manual is necessary for successful use of the chemagic™ Viral DNA/RNA 300 Kit H96. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers.
2. Do not use kit reagents after the expiry date printed on the kit label. Once opened, the reagents can be used for the time period stated in the reagent listing of IFU.
3. Any deviation from the protocol may affect the results.
4. The reagents are automatically dispensed in whole rows and therefore the tip covers (Disposable Tips) should be used also in whole rows on each rod in contact with any reagent solution. It should also be noted that if partial plates are run, the solutions may not be sufficient for 960 extractions.
5. Opening the chemagic™ 360-D instrument door while the automated extraction run is ongoing, will terminate the run and the samples in process may be lost.
6. Cleaning and maintenance of the system is described in detail in the chemagic™ 360-D User Manual.
    a. The system cleaning is performed once per week: Clean the chemagic™ Dispenser. Select the protocol "**regular cleaning procedure 96 dispenser 360 V150116.che**" and press [Insert IDs] or [Start] if the enhanced functions are deactivated. Follow the instructions as given in the software.
    b. Prior to the next use of the chemagic™ Dispenser perform the appropriate priming protocol.
    c. The cleaning of the chemagic™ Dispenser with 70 % ethanol is recommended once per month. Simply use the "**intensive cleaning procedure H96 dispenser 360 V150116.che"**' instead of the regular one for this purpose.
    d. If the chemagic™ Dispenser will not be used for longer period of time, it is mandatory to perform the "regular cleaning procedure" to maintain the performance of the instrument when bringing it back into service.

## PERFORMANCE NOTES 18 MIN PROTOCOL (ONLY TESTED WITH SARS-COV-2 ISOLATION)

For comparison of the 60 minutes protocol and the 18 minutes protocol extractions were performed using AccuPlex™ SARS-CoV-2 Reference Material (https://www.seracare.com/AccuPlex-SARSCoV2-Reference-Material-Kit-0505-0126/) spiked into transport medium from eNAT™ collection devices (Copan Italia S.p.A.) as sample material. The qPCR performance was tested with the EURORealTime SARS-CoV-2 qPCR (EUROIMMUN a PerkinElmer® company; kit used according to manufacturer instructions) run on a QuantStudio™ 5 Real-Time PCR System (96-well, 0.2 mL, desktop, Applied Biosystems™, A28574). The 18 minutes protocol for SARS-CoV-2 ("**chemagic Viral300 360 H96 prefilling 18 min VD210204.che**") gives the users the chance to triple their daily COVID-testing capacities. This shorter protocol can be used without any modifications or calibrations on the chemagic™ 360-D instrument, however the X-offset Bead Collection under Parameter Settings in the chemagic™ software must be disabled by a service engineer from PerkinElmer®. If the X-offset Bead Collection is enabled, the duration of the extraction run is elongated to 21 min. There is just a Ct value shift of 0.5 – 1 Ct compared to the 60 minutes standard protocol. Thus, the sensitivity is hardly reduced although there is a huge runtime and throughput benefit.

## DOWNSTREAM APPLICATIONS TESTED WITH SARS-COV-2 EXTRACTION

The following downstream applications were successfully performed and described in literature after isolation of SARS-CoV-2 samples with the chemagic™ Viral DNA/RNA 300 Kit H96 (CMG-1033-S).

| Downstream Application | Kits | Reference |
|---|---|---|
| RT-qPCR | TaqPath COVID-19 Combo Kit (Applied Biosystems™) | Barrett *et al.* BMC Infectious Diseases (2020) 20:853 https://doi.org/10.1186/s12879-020-05587-2 |
| | | Radbel *et al.* Journal of Molecular Diagnostics (2020) Volume 22, Issue 7, 871-875 https://doi.org/10.1016/j.jmoldx.2020.04.209 |
| | SuperScript™ III One-Step RT-PCR System with Platinum™ TaqDNA Polymerase (ThermoFisher) | Streeck *et al. Nat Commun* (2020) **11,** 5829 https://doi.org/10.1038/s41467-020-19509-y |

| Downstream Application | Kits | Reference |
|---|---|---|
| RT-qPCR | virella SARS-CoV-2 seqc rRT-PCR kit (Gerbion) | Wandernoth *et al.* Viruses (2020) 12:849 https://doi:10.3390/v12080849 |
| | 2019-nCoV CDC EUA Kit (IDT) | Xie *et al.* Processes (2020) *8*(11), 1425 https://doi.org/10.3390/pr8111425 |
| | SARS-CoV-2 real-time RT-PCR assay CE-IVD (PerkinElmer®) | Klussmeier *et al.* Biospektrum (2020) **26**, 500-503 https://doi.org/10.1007/s12268-020-1431-1 |
| | NeoPlex COVID-19 kit (Gene Matrix) | Senok *et al.* Infect Drug Resistance (2020) **13**, 3393-3399 https://doi.org/10.2147/IDR.S275152 |
| | NxTAG® Respiratory Pathogen Panel (Luminex Corporation), Fast Virus 1-Step Master Mix (ThermoFisher) | Kanji *et al.* Journal of the Association of Medical Microbiology and Infectious Disease Canada (2021) **1**, 10-15 https://doi.org/10.3138/jammi-2020-0035 |
| | 1) TRUPCR SARS-CoV-2 (Black Bio Biotech)<br>2) TaqPath RT-PCR COVID-19 Kit (ThermoFisher)<br>3) Allplex 2019-nCOV Assay (Seegene)<br>4) Patho detect COVID-19 qualitative PCR kit (My Lab)<br>5) LabGun COVID-19 RT-PCR Kit<br>6) Fosun COVID-19 RT-PCR detection kit (Fosun Ltd)<br>7) Realtime Fluorrescent RT-PCR kit (BGI Genomics) | Garg *et al.* Journal of Medical Virology (2021) **93**, 2281-2286 https://doi.org/10.1002/jmv.26691 |
| | Ligh™ix® Sarbeco V E-gene plus EAV control (TIB MolBiol) LightCycler® Multiplex RNA Virus Master (Roche) | Kriegshäuser *et al.* Clinical Chemistry and Laboratory Medicine (CCLM) (2021) **9**, 351-353 https://doi.org/10.1515/cclm-2021-0078 |

| Downstream Application | Kits | Reference |
|---|---|---|
| Sequencing | ARTIC V3 protocol | Kanji *et al.* Journal of the Association of Medical Microbiology and Infectious Disease Canada (2021) **1**, 10-15 https://doi.org/10.3138/jammi-2020-0035 |
| | | Jonsson *et al.* Nature Communications (2021) **12**, 3633 https://doi.org/10.1038/s41467-021-23883-6 |
| | | Tegally *et al.* Nature Medicine (2021) **27**, 440-446 https://doi.org/10.1038/s41591-021-01255-3 |
| | **cDNA synthesis:** LunaScript RT Super Mix kit (New England Biolabs), SuperScriptIV (ThermoFisher)<br>**Library prep.:** SureSelectXT Low Input kit CoVHuman6X enrichment capture-based method (Agilent Technologies)<br>ARTIC tiled amplicon multiplex PCR protocol (v3) + NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) | Ellingford *et al.* eLife (2021) **10**, 65453 https://doi.org/10.7554/eLife.65453 |

## FURTHER QUESTIONS

For further application, technical questions, or more information on how the data was generated please contact support.chemagen@Perkinelmer.com or +49 (0) 2401805500.

## LIMITATIONS OF THE PROCEDURE

The following collection devices are **not recommended** for use with the chemagic™ Viral DNA/RNA 300 Kit H96, for further questions please reach out to support.chemagen@perkinelmer.com.

| Description | Brand | Reference No. |
|---|---|---|
| Inactivated virus sampling tube (10 mL), containing 3 mL preservation medium (inactivated), 1x oropharyngeal swab with rayon material | Biocomma Limited | YMJ-TE |
| Virus collection and preservation system inactivated | Jiangsu Kangjian Medical Apparatus Co., Ltd. | KJ502-19C/D |

The performance characteristics of this product have not been established.

In some cases, traces of Magnetic Beads may be left in the eluate. Though such particles will usually not interfere with PCR or most downstream applications, an additional separation step either using centrifugation or a magnetic separator (chemagic™ Stand 96, provided with the chemagic™ 360 96 Rod Head Set, prod. no. CMG-370) is recommended, in order to separate any traces of particles.

Extracted DNA/RNA should be used immediately after extraction in the desired *in vitro* diagnostic test.

## WARRANTY

Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event PerkinElmer® chemagen Technologie GmbH and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

PerkinElmer® chemagen Technologie GmbH, its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

September 2022