INSTRUCTION FOR USE

chemagic™
BBS DNA Kit H12

Product number: **IVD-704**

Reagents for 250 extractions.

UDI-DI: 4260543364199

Version: V231026 EN

Manufacturer: Revvity chemagen Technologie GmbH
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CE

FOR *IN VITRO* DIAGNOSTIC USE.

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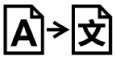
2. EXPLANATION OF THE SIGNAL WORDS IN THIS IFU

Signal word	Description
CAUTION!	Potential hazard that could lead to slight or medium harm.
ATTENTION!	Improper handling can damage the instrument.
NOTE:	Errors committed by the operator can cause that the optimal performance of the kit cannot be guaranteed.

3. SYMBOLS USED IN THE IFU AND ON LABELS

Symbol	Symbol Title	Symbol	Symbol Title
	CE mark European conformity		Temperature limit
	*In vitro* medical device		Contains sufficient for <n> tests
	Consult instructions for use or electronic instructions for use		Quantity
	Manufacturer		Do not re-use
	Batch code		Translation
	Catalogue number		Use-by date
	Do not use if package is damaged and consult instructions for use		This way up

Symbol	Symbol Title	Symbol	Symbol Title
	GHS02		Dangerous goods: Class 3 Flammable liquid
	GHS07		Dangerous goods: Class 8 Corrosive substances
	GHS08	-	-

4. APPLICATION

The chemagic™ BBS DNA Kit H12 is to be used for the automated extraction and purification of DNA from human blood, buffy coat and saliva using the chemagic™ 360-D instrument.

Other sample materials such as swabs or tissue lysates may be compatible but have not yet been validated. For such materials, a validation must be performed by the user.

The kit is designed to be used with IVD downstream applications employing enzymatic amplification and detection of DNA (e.g. PCR, RT-PCR, NGS). The product is intended for trained laboratory personnel and especially trained for the chemagic BBS DNA Kit H12 and the chemagic 360-D instrument.

For further information please refer to the sections “KIT REAGENTS AND SAFETY INFORMATION“ and “WARNINGS AND PRECAUTION” in this document.

5. SUMMARY AND PRINCIPLE

The chemagic BBS DNA Kit H12 is based on a magnetic bead technology platform proprietary to Revvity chemagen Technologie GmbH. White blood cells or other source of DNA present in blood, buffy coat and saliva 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 with a chemagic 12 Rod Head Set 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.

6. REPORTING OF INCIDENTS

For a 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 Revvity chemagen Technologie GmbH, +49 (0) 2401805500 or support.chemagen@revvity.com or it´s legal representatives.

The competent authority in Germany is the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM). Current contact information can be found on the BfArM website: https://www.bfarm.de.

7. GENERAL AND STORAGE INFORMATION

The kit contains reagents sufficient to perform 250 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 (section “KIT REAGENTS AND SAFETY INFORMATION”).

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.

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 12, provided with the chemagic 360 12 Rod Head Set) is recommended, in order to separate any traces of particles.

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

In this IFU we refer to the chemagic 360-D User Manual. This manual will be provided with the chemagic 360-D instrument.

Kit-related protocol files are available on the webpage or will be provided by customer support (see section “REQUIRED PROTOCOLS FILES”).

8. 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/products/ce-ivd-chemagic-kits/ivd-704-chemagic-bbs-dna-kit-h12/.

The eIFU are provided in at least English (EN), French (FR), Spanish (ES) and Italian (IT) and upon request also in other required languages.

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

9. WARNINGS AND PRECAUTION

For *in vitro* diagnostic use.

The product is intended for professional users trained for the chemagic 360-D instrument.

A thorough understanding of this IFU and the chemagic 360-D User Manual is a prerequisite and necessary for successful use of the chemagic BBS DNA Kit H12.

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.

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.

Any deviation from the protocol may affect the results.

The reagents are automatically dispensed in whole rows and therefore the chemagic Tips XL and the 50 mL Tubes 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 250 extractions.

Check all kit components for integrity. In case of damage, contact your supplier.

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 chloride and is harmful if swallowed, in contact with skin or if inhaled. Binding Buffer 2, Wash Buffer 3 and Wash Buffer 4 contain sodium perchlorate and ethanol and are flammable liquids and vapors and are harmful if swallowed. Wash Buffer 5 contains ethanol and is a flammable liquid and vapor. Proteinase K contains Tritirachium album serine Proteinase and causes skin irritation and serious eye irritation. It may cause allergy or asthma symptoms or breathing difficulties or respiratory irritation if inhaled. See specific precautions for all components in the section “KIT REAGENTS AND SAFETY INFORMATION”.

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.

10. KIT REAGENTS AND SAFETY INFORMATION

The chemagic BBS DNA Kit H12 contains the following reagents.

10.1 MAGNETIC BEADS

Component	Quantity	Shelf life and storage
Magnetic Beads	1 bottle (volume see label)	+2 to +25 °C until expiry date stated on the bottle label. 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 during the extraction process.

10.2 LYSIS BUFFER 1

Component	Quantity	Shelf life and storage
Lysis Buffer 1  WARNING	1 canister (volume see label)	+2 to +25 °C until expiry date stated on the canister label. Once opened, stable for 60 days at +2 to +25 °C.

Ready-for-use aqueous buffer (pH 6.7-7.2) solution containing guanidinium chloride (30–50 %) and isotridecyl alcohol (1-1.5 %). Lysis Buffer 1 is used to lyse the cells or other DNA source present in the sample to release DNA in solution.

CAUTION! Lysis Buffer 1 contains guanidinium chloride and isotridecyl alcohol.

Hazard, precautionary and EUH phrases	
H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Hazard, precautionary and EUH phrases	
P301+P312	IF SWALLOWED: Call a POISON/ doctor if you feel unwell. P330 Rinse mouth.
P330	Rinse mouth.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313	If skin irritation occurs: Get medical advice/ attention.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

10.3 BINDING BUFFER 2

Component	Quantity	Shelf life and storage
Binding Buffer 2  DANGER	4 canisters (volume see label)	+2 to +25 °C until expiry date stated on the canister label. Once opened, stable for 60 days at +2 to +25 °C.

Ready-for-use Tris-HCl-buffered (pH 5.2–5.9) solution with sodium perchlorate (20–30 %), ethanol (30–50 %) and acetic acid (0.75–1.5 %). Binding Buffer 2 is used to create the appropriate conditions to get the DNA bound to the Magnetic Beads.

CAUTION! Binding Buffer 2 contains ethanol and sodium perchlorate.

Hazard, precautionary and EUH phrases	
H226	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.

Hazard, precautionary and EUH phrases	
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.

10.4 WASH BUFFER 3

Component	Quantity	Shelf life and storage
Wash Buffer 3  DANGER	2 canisters (volume see label)	+2 to +25 °C until expiry date stated on the canister label. Once opened, stable for 60 days at +2 to +25 °C.

Ready-for-use Tris-HCl-buffered (pH 5.0–5.6) solution with sodium perchlorate (10–20 %) and ethanol (10–30 %). Used for removing non-DNA contaminants during washing step.

CAUTION! Wash Buffer 3 contains ethanol and sodium perchlorate.

Hazard, precautionary and EUH phrases	
H226	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.

10.5 WASH BUFFER 4

Component	Quantity	Shelf life and storage
Wash Buffer 4  DANGER	2 canisters (volume see label)	+2 to +25 °C until expiry date stated on the canister label. Once opened, stable for 60 days at +2 to +25 °C.

Ready-for-use Tris-HCl-buffered (pH 5.0–5.6) solution with sodium perchlorate (10–20 %) and ethanol (10–30 %). Used for removing non-DNA contaminants during washing step.

CAUTION! Wash Buffer 4 contains ethanol and sodium perchlorate.

Hazard, precautionary and EUH phrases	
H226	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.

10.6 WASH BUFFER 5

Component	Quantity	Shelf life and storage
Wash Buffer 5  DANGER	2 canisters (volume see label)	+2 to +25 °C until expiry date stated on the canister label. 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 contaminants during washing step.

CAUTION! Wash Buffer 5 contains ethanol.

Hazard, precautionary and EUH phrases	
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.

10.7 WASH BUFFER 6

Component	**Quantity**	**Shelf life and storage**
Wash Buffer 6	2 canisters (volume see label)	+2 to +25 °C until expiry date stated on the canister label. 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.

10.8 ELUTION BUFFER 7

Component	Quantity	Shelf life and storage
Elution Buffer 7	2 bottles (volume see label)	+2 to +25 °C until expiry date stated on the bottle label. 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.

10.9 PROTEINASE K

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

Proteinase K is reconstituted by adding 7 mL of purified water. Proteinase K is added to enhance the efficiency of the lysis step.

CAUTION! Proteinase K contains Proteinase, Tritirachium album serine and calcium acetate hydrate.

Hazard, precautionary and EUH phrases	
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.

Hazard, precautionary and EUH phrases	
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.

10.10 FURTHER KIT COMPONENTS

The chemagic BBS DNA Kit H12 contains the following plastic material.

Component	Quantity	Storage
chemagic Tips XL	250	+2 to +25 °C

11. REQUIRED PROTOCOLS FILES

The following protocol files will be provided by Revvity chemagen Technologie GmbH and are available on the webpage or will be provided by customer support.

Protocol (.che file)	Protocol type/ purpose
chemagic BBS DNA 360 H12 EB50 drying prefilling VD220308.che	Kit-related extraction file for the chemagic 360-D instrument
prime manifolds H12 all 360 V150116.che	Filling and priming the chemagic 360-D instrument tubing with reagents
check manifolds H12 all 360 V150116.che	Checking the functionality of the pumps
regular cleaning procedure H12 dispenser 360 V150116.che	Regular cleaning of the chemagic 360-D instrument (once per week)
intensive cleaning procedure H12 dispenser 360 V150116.che	Intensive cleaning of the chemagic 360-D instrument (once per month)

12. MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

The chemagic BBS DNA Kit H12 requires the following items.

12.1 ITEMS FROM REVVITY CHEMAGEN TECHNOLOGIE GMBH

Item	Product no.
chemagic 360-D instrument	2024-0010
chemagic 12 Rod Head Set	CMG-371
Racks for 50 mL tubes (provided with chemagic 12 Rod Head Set)	-

12.2 ADDITIONAL REQUIRED ITEMS

Item	Purpose
50 mL Conical Centrifuge Tubes (e. g. Falcon® Tubes) 72x / extraction	Reaction vessel
Pipettes and pipette tips with aerosol barriers	Prefilling of Magnetic Beads, Elution Buffer 7 and Proteinase K
Molecular biology grade water	Reconstitution of the Proteinase K
70% Ethanol	Cleaning of the chemagic 360-D instrument

12.3 ADDITIONAL OPTIONAL ITEMS FROM REVVITY CHEMAGEN TECHNOLOGIE GMBH

Product	Product no.
chemagic Stand 12 (supplied with the chemagic 12 Rod Head Set)	CMG-308
Red Cell Lysis Buffer	CMG-848

12.4 OTHER ADDITIONAL OPTIONAL ITEMS

Product	Purpose
Isotonic saline solution, sterile	Resuspension of buffy coats

13. SPECIMEN COLLECTION AND HANDLING

The chemagic BBS DNA Kit H12 is usable with human blood, buffy coat and saliva in aliquots of up to 10 mL per isolation.

Human whole blood (up to 10 mL) or buffy coat samples (up to 10 mL) that are fresh, frozen or stored typically for a maximum of 10 days at +2 to +8 °C should be used. For long-term storage, freezing at –20 °C or –80 °C in aliquots is recommended. The recommended blood stabilizers are EDTA or citrate.

NOTE: The use of heparin-stabilized blood samples can cause inhibition in downstream applications and is therefore not recommended.

The white blood cell count in the whole blood sample decreases during storage. Prolonged storage of the samples may cause a poor yield of the DNA after extraction.

Human saliva (10 mL) stored according to the collection tube provider instructions should be used. The recommended stabilized collection tubes are from DNAgenotek®, Isohelix™ and Spectrum Solutions. Incubation of the collection tubes prior to the extraction for > 2 h at 50 °C will result in higher DNA yields and is therefore recommended.

Buffy coat samples stored for a maximum of one week at +2 to +8 °C should be used. For long-term storage, freezing at –20 °C or –80 °C in aliquots is recommended. The buffy coats should be derived from stabilized blood tubes (follow tube recommendation above for blood samples). Prior to the extraction, buffy coats should be thawed at 37 °C. We recommend the following procedure for the buffy coat preparation.

14. PREPARATION OF BUFFY COAT SAMPLES

- Place up to 5 mL of fresh whole blood into a sterile 50 mL tube (prepare two buffy coats samples for each extraction sample).
- Add 40 mL Red Cell Lysis Buffer (RCLB) to the blood and invert the tube 4 times.
- Incubate for 5 minutes or until the suspension becomes translucent.
- Centrifuge at 4,000 rpm for 10 minutes to collect the white blood cells.
- Decant the supernatant and carefully aspirate the remaining supernatant from the top of the sample by pipetting.

NOTE: Be careful not to disturb the cell pellet. Exercise caution in pipetting to avoid loss of the white blood cell pellet.

- Add 20 mL Red Cell Lysis Buffer and carefully wash and remove remaining red cells on top of the white pellet without disturbing the white pellet.
- Decant the supernatant and carefully aspirate the remaining supernatant from the top of the sample by pipetting.
- Resuspend the buffy coat in up to 2 mL isotonic saline solution (0.9 % NaCl).
- If the starting volume of blood is less or more than 5 mL, change the volume of Red Cell Lysis Buffer used proportionately.
- The buffy coats can be frozen, stored at +2 to +8 °C for one week, or directly used for extraction.

15. DETAILED PROTOCOL DESCRIPTION

15.1 PROTOCOL PROCEDURE

The following procedure describes the preparation and the execution of the extraction protocol using the chemagic 360-D instrument.

The duration of the automated extraction protocol is approximately 78 minutes.

The protocol is suitable for processing up to 12 samples in parallel (see “PROCESSING STEPS” below). For detailed instructions on the use of the chemagic 360-D instrument, please refer to the chemagic 360-D User Manual.

NOTE: 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	Buffer	Minimum filling volume
Pump 1	Lysis Buffer 1	175 mL
Pump 2	Binding Buffer 2	400 mL
Pump 3	Wash Buffer 3	250 mL
Pump 4	Wash Buffer 4	250 mL
Pump 5	Wash Buffer 5	250 mL
Pump 6	Wash Buffer 6	150 mL

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, Wash Buffer 4 and Wash Buffer 5 contain ethanol. If ethanol evaporates, the optimal yield or detection sensitivity cannot be guaranteed.

15.2 PROCESSING STEPS

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. Load the 50 mL tubes in the racks for 50 mL tubes.
3. Before prefilling the 50 mL tubes in the racks mark them with material in position (samples, Magnetic Beads and buffers).

   The reagents are automatically dispensed in whole rows and therefore the 50 mL tubes should be used also in whole rows on each rod in contact with any reagent solution.
4. Reconstitute the Proteinase K:

Component	Reconstitution
Proteinase K	Add 7 mL molecular biology grade water to Proteinase K bottle and mix gently until dissolved.

5. Fill and prime the chemagic 360-D tubing with reagents by choosing the protocol "**prime manifolds H12 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].

NOTE: 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 H12 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 BBS DNA 360 H12 EB50 drying prefilling VD220308.che**" and press [Insert IDs] and follow the instructions given in the chemagic QA software.
8. Ensure chemagic Tips XL are sufficient and aligned with the positions of the samples and place the Tip Rack in position 1 on the tracking system. The reagents are automatically dispensed in whole rows and therefore the

chemagic Tips XL should be used also in whole rows on each rod in contact with any reagent solution.

9. Check the volumes in the buffer supply containers and confirm by pressing [OK]. See above “Protocol PROCEDURE” minimum filling heights.

NOTE: Take care that all buffer supply bottles contain enough buffer. Only if the liquid level for all buffers is sufficient 12 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 tubes with up to 10 mL sample. To ensure the homogeneity of the samples, mix the samples gently prior to pipetting in the sample tubes.
12. Prefill Elution Buffer 7 and the thoroughly resuspended Magnetic Beads by pipetting manually according to each corresponding tube in use.

Component	Plate position on chemagic 360-D instrument	Volume/ well or tube
Magnetic Beads	3	900 µL
Elution Buffer 7	7	1.2 – 1.5 mL

NOTE: The Magnetic Bead suspension should be mixed vigorously before dispensing; otherwise the suspension is not homogenous and the DNA yield could be low.

13. Add 50 µL Proteinase K to the tubes containing sample.
14. Place the racks on the tracking system according to the instructions given by the chemagic QA software.
15. Place the sample rack in position 2 on the tracking system.
16. Check all tubes and racks for accurate orientation and fitting.
17. Close the front door and start the process by pressing [Start].
18. The automated DNA extraction process is initiated.

19. 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.

ATTENTION! Never move the tracking system (table) manually. This might damage the instrument. All movements must be performed with the [Turn Table] function.

NOTE: 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.

For information on cleaning the instrument see section “CLEANING AND MAINTENANCE”.

15.3 SHORT DESCRIPTION/ QUICK GUIDE

Automated DNA extraction run on chemagic 360-D instrument:

- Select the protocol "**check manifolds H12 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 BBS DNA 360 H12 EB50 drying prefilling VD220308.che**" and press [Insert IDs]. Follow the instructions given in the chemagic QA software to fill in the required data.
- Load the racks with 50 mL tubes.
- The reagents are automatically dispensed in whole rows and therefore the chemagic Tips XL and the 50 mL tubes should be used also in whole rows on each rod in contact with any reagent solution.
- Load the racks on the tracking system positions 1-8 as follows. (Numbers on tracking system refer to the positioning of the plate on the chemagic 360-D instrument.)

Position on tracking system	Material in position	Protocol step in detail
1	Rack with chemagic Tips XL	Use chemagic Tips XL according to the positions of the samples and place the rack. **Note: Tips need to be present in the rack in full rows.**
2	Sample rack (Rack with 50 mL tubes)	Place the rack with prepared samples (up to 10 mL sample) and 50 µL Proteinase K. Lysis Buffer 1 and Binding Buffer 2 are dispensed in the tubes automatically.
3	Rack with 50 mL tubes with 900 µL Magnetic Beads	Pipette 900 µL thoroughly resuspended Magnetic Beads in each tube in use according to the sample tubes and place the rack. Wash Buffer 3 is dispensed in the tubes automatically.
4	Rack with 50 mL tubes	Place rack with empty 50 mL tubes. Wash Buffer 4 is dispensed in the tubes automatically.
5	Rack with 50 mL tubes	Place rack with empty 50 mL tubes. Wash Buffer 5 is dispensed in the tubes automatically.
6	Rack with 50 mL tubes	Place rack with empty 50 mL tubes. Wash Buffer 6 is dispensed in the tubes automatically.
7	Rack with 50 mL Tubes prefilled with 1.2 – 1.5 mL Elution Buffer 7	Pipette 1.2 – 1.5 mL Elution Buffer 7 to each 50 mL tube according to the sample positions and place the rack.
8	empty	-

- Check all tubes and racks for accurate orientation and fitting.
- After all tubes and racks are in place, press [OK].
- Close the front door and start the DNA extraction process immediately by pressing [Start]. Subsequently the sample lysate will be mixed automatically.
- If the functions enabling the ID data input are deactivated, load the plates on the tracking system positions 1-8.
- After all plates are in place, select the protocol "**chemagic BBS DNA 360 H12 EB50 drying prefilling VD220308.che**", mark the columns in use on the plate map in the dialog and start the extraction run directly by pressing [Start].
- 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.

ATTENTION! Never move the tracking system (table) manually. This might damage the instrument. All movements must be performed with the [Turn Table] function.

NOTE: 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.

16. PERFORMANCE CHARACTERISTICS

16.1 LINEARITY AND RECOVERY WITH SPIKED DNA SAMPLE

To show linearity of the extraction with the IVD-704 kit, 0.9 % NaCl solution was spiked with five concentrations of human genomic DNA; 100 µg, 150 µg, 200 µg, 300 µg and 400 µg. Extractions were performed using 10 mL of the various DNA amounts as sample with the extraction protocol "**chemagic BBS DNA 360 H12 EB50 drying prefilling VD220308.che**". Four replicates of each DNA amount spiked in 0.9% NaCl were extracted.

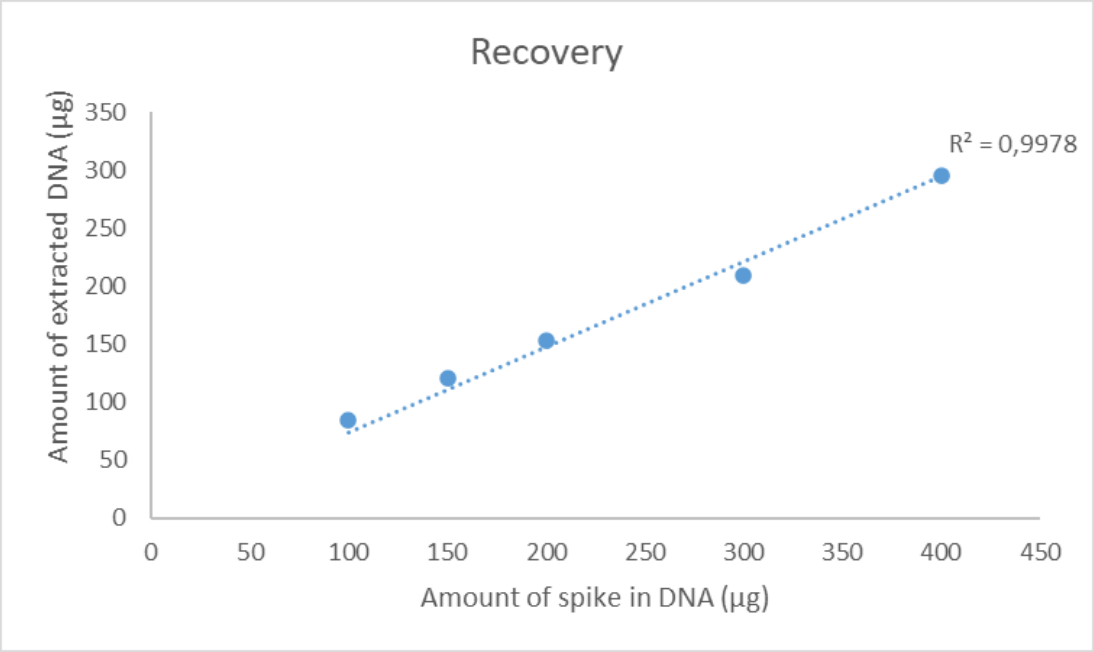


Figure 1: Extraction recovery of spike in DNA samples. Extracted with the chemagic 360-D using the IVD-704 kit.

The recovery shows a good linearity in the range of 100 – 400 µg of DNA as sample input. The recovery ranges between 69.8 and 84.6 % depending on the input amount of human genomic DNA.

16.2 DNA YIELDS WITH BLOOD AND BUFFY COAT SAMPLES

The expected DNA yields for the extraction from human blood and buffy coat is depending on the number of white blood cells. The number of the extracted white blood cells is determined by the input volume and the white blood cell count (WBC). For most of the samples the white blood cell count will not be known, but for healthy individuals it is in the range of 4 – 10 mio. white blood cells per mL of blood. The IVD-704 kit using the "**chemagic BBS DNA 360 H12 EB50 drying prefilling VD220308.che**" protocol extracts in average 4.38 pg per white blood cell. Using 10 mL of blood with a white blood cell count of 8.2 mio. white blood cells per mL of blood as input is expected to result in 359 µg DNA.

Table 1: Average yield, %CV (standard deviation) and purity of extracted DNA using the IVD-704 kit extracted with the chemagic 360-D.

Sample Material / Storage Condition	Volume [mL]	WBC [mio. cells/ mL Blood]	Average Yield [µg]	CV [%]	Average Purity [260/280]
Blood 1 / 4 °C	10	4.9	220.7	1.9	1.9
Blood 1 / -20 °C	10	4.9	238.7	10.1	1.9
Blood 1 / 4 °C	5	4.9	117.6	3.9	1.9
Blood 2 / 4 °C	10	5.8	286.3	4.4	1.9
Blood 2 / -20 °C	10	5.8	305.0	4.7	1.9
Blood 2 / 4 °C	5	5.8	150.3	4.9	1.9
Blood 4 / 4 °C	10	6.1	322.0	6.2	1.9
Blood 5 / 4 °C	10	9.3	509.1	5.6	1.9
Buffy Coat*	10	6.1	237.0	2.8	1.8
Saliva / 4 °C	10	-	128.1	11.5	1.6

* Buffy Coat was generated from 10 mL of Blood 3

From all extractions including different sample materials, storage conditions and input volumes, specific eluates were used in a human albumin qPCR to prove the suitability of the extracted DNA for enzymatic reactions. All eluates performed without any issues - see example qPCR plot below.

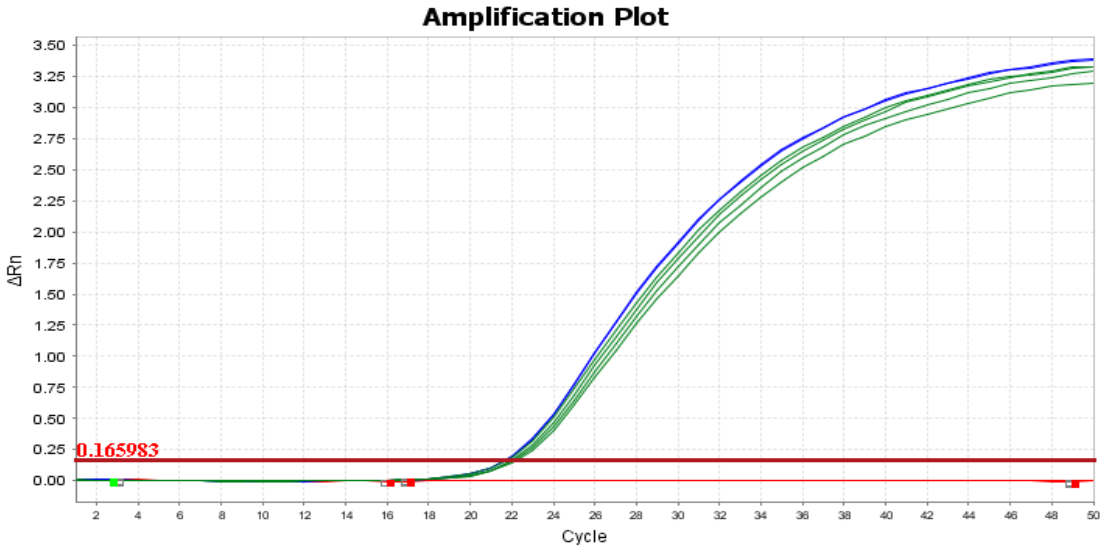


Figure 2: qPCR Curves of DNA eluates extracted with the chemagic 360-D using the IVD-704 kit. Red - negative control, blue – positive control, green extracted DNA from Blood 1.

17. CLEANING AND MAINTENANCE

Cleaning and maintenance of the system is described in detail in the chemagic 360-D User Manual. The system cleaning is performed once per week. Clean the chemagic Dispenser as follows.

- Select the protocol "**regular cleaning procedure H12 dispenser 360 V150116.che**" and press [Insert IDs] or [Start] if the enhanced functions are deactivated. Follow the instructions as given in the software.
- Prior to the next use of the chemagic Dispenser perform the appropriate priming protocol.
- The cleaning of the chemagic Dispenser with 70 % ethanol is recommended once per month. Simply use the "**intensive cleaning procedure H12 dispenser 360 V150116.che**" instead of the regular one for this purpose.
- 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.

18. DOWNSTREAM APPLICATIONS

The following downstream applications were successfully performed and described in literature after isolation of genomic DNA.

Table 2: Peer revied and published downstream applications.

Sample Material	Downstream Application	Title	Reference
frozen human whole blood	SNP Genotyping	No Association between Genetic Loci near IRF2 and TBX1 and Acute Kidney Injury in the Critically Ill	American Journal of Respiratory and Critical Care Medicine (2019-09) https://www.atsjournals.org/doi/10.1164/rccm.201903-0633LE
blood	NGS/Sanger Sequencing	Identification of a novel mutation in the PRCD gene causing autosomal recessive retinitis pigmentosa in a Turkish family	Molecular Vision (2013-06) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3692407/
blood and tissue sections	Multiplex PCR	Cell lines authentication and mycoplasma detection as minimun quality control of cell lines in biobanking	Cell and Tissue Banking (2017-03) https://link.springer.com/article/10.1007/s10561-017-9617-6
whole blood	UV-measurement, Sequencing, MLPA	Genetic architecture of inherited retinal degeneration in Germany: A large cohort study from a single diagnostic center over a 9-year period	Human Mutation (2020-06) https://onlinelibrary.wiley.com/doi/full/10.1002/humu.24064
whole blood (frozen, -80°C)	UV-measurement, Genotyping: PCR + Pyrosequencing	Mechanisms and modulators of cognitive training gain transfer in cognitively healthy aging: study protocol of the AgeGain study	Trials (2018-06) https://trialsjournal.biomedcentral.com/articles/10.1186/s13063-018-2688-2

Sample Material	Downstream Application	Title	Reference
peripheral venous blood	Genotyping	Positive Association between TGFB1 Gene and Susceptibility to Idiopathic Scoliosis in Bulgarian Population	Analytical Cellular Pathology (2018-07) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6069583/
peripheral venous blood	Genotyping	Positive association between a polymorphic locus near the LBX1 gene and predisposition of idiopathic scoliosis in Southeastern European population	Journal of Applied Biomedicine (2019-06) https://pubmed.ncbi.nlm.nih.gov/34907700/
venous blood	UV-measurement, picogreen measurement, HLA typing	In silico analysis of HLA associations with druginduced liver injury: use of a HLA-genotyped DNA archive from healthy volunteers	Genome Medicine (2012-06) https://genomemedicine.biomedcentral.com/articles/10.1186/gm350
peripheral blood	SNP Genotyping	Single Nucleotide Polymorphisms in Colorectal Cancer: Associations with Tumor Site and TNM Stage	Journal of gastrointestinal and liver diseases (2012-01) https://pubmed.ncbi.nlm.nih.gov/22457859/
blood	DNA genotyping and imputation	Genetic regulatory effects modified by immune activation contribute to autoimmune disease associations	Nature Communications (2017-08) https://www.nature.com/articles/s41467-017-00366-1
blood	agarose gel, methylation analysis	DNA methylation levels and long-term trihalomethane exposure in drinking water: an epigenome-wide association study	Epigenetics (2015-06) https://www.tandfonline.com/doi/full/10.1080/15592294.2015.1057672
blood cultures	PCR + SNP genotyping + Sanger Sequencing	Human Genetic Susceptibility to Native Valve Staphylococcus aureus Endocarditis in Patients with	Frontiers in Microbiology (2018-04) https://www.frontiersin.org/ar

Sample Material	Downstream Application	Title	Reference
		S.aureus Bateremia: Genome-Wide Association Study	ticles/10.3389/fmicb.2018.00640/full
venous blood	genetic analysis	Mutation spectrum of the OPA1 gene in a large cohort of patients with suspected dominant optic atrophy: Identification and classification of 48 novel variants	PLOS ONE (2021-07) https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0253987
peripheral blood	Single SNP Genotyping	Replication study of 34 common SNPs associated with prostate cancer in the Romanian population	Journal of Cellular and Molecular Medicine (2016-05) https://onlinelibrary.wiley.com/doi/10.1111/jcmm.12729
blood samples (after separation of plasma)	genotyping (PCR and capillary sequencing)	Heme oxygenase-1 repeat polymorphism in septic acute kidney injury	PLOS ONE (2019-05) https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0217291
whole blood	Methylation arrays	The role of environmental stress and DNA methylation in the longitudinal course of bipolar disorder	International Journal of Bipolar Disorders (2020-02) https://journalbipolardisorders.springeropen.com/articles/10.1186/s40345-019-0176-6

19. FURTHER QUESTIONS

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

20. LIMITATIONS OF THE PROCEDURE

The IVD-704 kit is validated for the extraction of DNA from blood, buffy coat and saliva. Other sample materials such as tissue, cell or buccal swab lysates may be compatible but have not been validated. For such materials, a validation must be performed by the user.

The use of heparin-stabilized blood samples can cause inhibition in downstream applications and is therefore not recommended.

21. INFLUENCE OF INTERFERING SUBSTANCES

The effect of interfering substances contained in human whole blood possibly interfering with the DNA extraction were tested in whole blood. The tested substances and concentrations are presented in table below. Based on the results, it was concluded that the tested substances do not interfere with the DNA extraction.

Table 3: Influence of interfering substances.

Interfering substances	Concentration [µg/mL]	Interference
Bilirubin conjugated	332	No
Bilirubin unconjugated	200	No
Triglycerides	30	No
Human Serum Albumin	30	No

22. WARRANTY

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

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