

3095-0030

chemagic™ DNA Blood200 kit

Instructions for use. Reagents for 960 extractions.

Manufacturer:

Wallac Oy

Mustionkatu 6, FI-20750 Turku, Finland

Phone: +358 2 2678 111

FOR *IN VITRO* DIAGNOSTIC USE

CE

revvity

SYMBOLS

In vitro diagnostic medical device



Batch code



Packing number



Catalogue number



Use-by date



Temperature limit



Contains sufficient for <n> tests



Consult instructions for use



Manufacturer



GHS07



GHS02



GHS08



This way up



Recyclable



Fragile, handle with care



Keep dry

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chemagic™ DNA Blood200 kit

INTENDED PURPOSE

The chemagic™ DNA Blood200 kit is intended for the extraction and purification of DNA from human whole blood for analysis by PCR based *in vitro* diagnostic test systems. The disorder and determination of analytes are dependent on the PCR based downstream assay. The product is intended to be used with an automated workflow by trained laboratory personnel.

SUMMARY AND PRINCIPLE

The chemagic DNA Blood200 kit is based on a magnetic bead technology platform proprietary to Revvity chemagen Technologie GmbH. Cells present in whole blood samples 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 (2024-0010) with chemagic 360 96 Rod Head Set (CMG-370) or equivalent instrument.

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 are stable for 60 days. 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.

The kit contains following items:

Component	Quantity
Magnetic Beads B	2 bottles, 150 mL
Lysis Buffer B	2 bottles, 480 mL
Binding Buffer B	2 bottles, 550 mL
Wash Buffer BB	2 bottles, 700 mL
Wash Buffer BA	2 bottles, 700 mL
Wash Buffer E	2 bottles, 700 mL
Wash Buffer H	2 bottles, 700 mL
Elution Buffer	1 bottle, 240 mL
Lot-specific quality control certificate	1 pc

Reagents

Component	Stability and storage
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Magnetic Beads B +2 to +25 °C until expiry date stated on the bottle label.

Suspension of particles containing nanoparticulate iron oxide encapsulated in a matrix of polyvinyl alcohol. Magnetic Beads (28.0 ± 0.5 mg/mL) bind the DNA during the extraction process.

Lysis Buffer B +2 to +25 °C until expiry date stated on the bottle label.
Avoid direct sunlight.



WARNING

Ready-for-use aqueous buffer solution (pH 6.9–7.4) containing guanidinium chloride (15–25 %) and detergent. Lysis Buffer is used to lyse the blood cells in order to get the DNA in solution.

Lysis Buffer B contains guanidinium chloride:

H315 Causes skin irritation.

H319 Causes serious eye irritation.

P264 Wash thoroughly after handling.

P280 Wear protective gloves / eye protection / face 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.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P362+P364 Take off contaminated clothing and wash it before reuse.

P337+P313 If eye irritation persists: Get medical advice/attention.

Binding Buffer B

+2 to +25 °C until expiry date stated on the bottle label.

**DANGER**

Ready-for-use Tris-HCl-buffered (pH 5.0–5.9) solution with sodium perchlorate (15–25 %) and ethanol (25–50 %). Binding Buffer is used to create the appropriate conditions to get the DNA bound to the Magnetic Beads.

Binding Buffer B contains sodium perchlorate and ethanol:

H225 Highly flammable liquid and vapor.

H319 Causes serious eye irritation.

H373 May cause damage to organs through prolonged or repeated exposure.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.

No smoking.

P241 Use explosion-proof [electrical/ventilating/lighting] equipment.

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.

P403+P235 Store in a well-ventilated place. Keep cool.

P501 Dispose of contents/container in accordance with local regulations.

Wash Buffer BB

+2 to +25 °C until expiry date stated on the bottle label.

**DANGER**

Ready-for-use Tris-HCl-buffered (pH 5.0–5.6) solution with sodium perchlorate (15–25 %) and ethanol (25–50 %). Used for removing non-DNA contaminants during washing step.

Wash Buffer BB contains sodium perchlorate and ethanol:

H225 Highly flammable liquid and vapor.

H319 Causes serious eye irritation.

H373 May cause damage to organs through prolonged or repeated exposure.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.

No smoking.

P241 Use explosion-proof [electrical/ventilating/lighting] equipment.

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.

P403+P235 Store in a well-ventilated place. Keep cool.

P501 Dispose of contents/container in accordance with local regulations.

Wash Buffer BA

+2 to +25 °C until expiry date stated on the bottle label.



DANGER

Ready-for-use Tris-HCl-buffered (pH 5.0–5.6) solution with sodium perchlorate (15–25 %) and ethanol (25–50 %). Used for removing non-DNA contaminants during washing step.

Wash Buffer BA contains sodium perchlorate and ethanol:

H225 Highly flammable liquid and vapor.

H319 Causes serious eye irritation.

H373 May cause damage to organs through prolonged or repeated exposure.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.

No smoking.

P241 Use explosion-proof [electrical/ventilating/lighting] equipment.

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.

P403+P235 Store in a well-ventilated place. Keep cool.

P501 Dispose of contents/container in accordance with local regulations.

Wash Buffer E

+2 to +25 °C until expiry date stated on the bottle label.



DANGER

Ready-for-use solution contains ethanol 50–75 %. Used for removing last traces of non-DNA contaminants during washing step.

Wash Buffer E contains ethanol:

H225 Highly flammable liquid and vapor.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.

No smoking.

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].

P403+P235 Store in a well-ventilated place. Keep cool.

P501 Dispose of contents/container in accordance with local regulations.

Wash Buffer H +2 to +25 °C until expiry date stated on the bottle label.
Ready-for-use ultra-filtered water solution. Used for removing possible residuals of ethanol.

Elution Buffer +2 to +25 °C until expiry date stated on the bottle label.
Ready-for-use 10 mM Tris-HCl-buffered (pH 7.8–8.4) solution.

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The chemagic DNA Blood200 kit requires the following items, which are available from Wallac Oy or Revvity, Inc. and its distributors:

- chemagic 360-D (prod. no. 2024-0010) with chemagic 360 96 Rod Head Set (prod. no. CMG-370) or equivalent instrument
- chemagic consumables (tips, deep well plates and low well plates for chemagic, prod. no. 4155-0010)

Additional required items:

- pipettes and pipette tips with aerosol barriers

Additional optional items:

- chemagic Stand 96 (prod. no. CMG-301)

SPECIMEN COLLECTION AND HANDLING

Human whole blood samples (200 µL), fresh or stored typically for a maximum of one week at +2 to +8 °C should be used. The recommended blood stabilizers are EDTA or citrate. The use of heparin stabilized blood samples can cause inhibition problems in PCR and is therefore not recommended.

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

The extraction efficiency using other types of sample material has not been determined.

Specimen Stability

The influence of storage time and temperature was studied¹ using several whole blood specimens collected from healthy donors to collection tubes containing either EDTA or citrate as an anticoagulant. Samples were stored at +2 to +8 °C up to 7 days. Extracted DNA samples were replicated in CE IVD-registered PCR assay and samples were tested by a commercially available downstream assay kit for detecting Fragile X syndrome using recommended reference samples by the National Institute for Biological Standards and Control (NIBSC). No clinically significant deviation was observed on results.

WARNINGS AND PRECAUTIONS

The product is intended to be used by trained laboratory personnel.

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, and sample amplification and detection according to the downstream assay used.

Handle all patient specimens as potentially infectious. Nevertheless all recommended precautions for the handling of blood derivatives should be observed. 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 B contains guanidinium chloride and causes skin irritation and serious eye irritation. **Wash Buffer BB, Wash Buffer BA and Binding Buffer B contain sodium perchlorate and ethanol** and are highly flammable liquids and vapors, cause serious eye irritation and may cause damage to organs through prolonged or repeated exposure. **Wash Buffer E contains ethanol** and is highly flammable liquid and vapor. See specific precautions from the section "KIT CONTENTS".

To avoid injuries 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.

For a patient/user/third party in the European Union and in countries with an identical regulatory regime (IVDR; EU 2017/746/EU); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and to your national authority. The contact information for the manufacturer of this device to report a serious incident is stated on the cover page of these instructions for use.

¹ Study performed at Wallac Oy, Turku, Finland.

PROCEDURE

Extraction protocol using the chemagic 360-D instrument

The duration of the automated extraction protocol is approximately 75 minutes.

The protocol is suitable for processing up to 96 samples in parallel (see protocol steps 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.

NOTE: Recap the bottles tightly immediately after use or keep the bottles connected tightly to the chemagic 360-D instrument. Binding Buffer B, Wash Buffer BB, Wash Buffer BA and Wash Buffer E contain ethanol. If ethanol evaporates, the optimal yield or detection sensitivity cannot be guaranteed.

Processing Steps in Detail

Preparing Steps

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. Connect the reagent bottles to chemagic 360-D instrument as follows:
 - Manifold 1: No bottle connected
 - Manifold 2: No bottle connected
 - Manifold 3: Wash Buffer BB
 - Manifold 4: Wash Buffer BA
 - Manifold 5: Wash Buffer E
 - Manifold 6: Wash Buffer H
3. Fill and prime the chemagic 360-D tubing with reagents by choosing protocol '**prime manifolds H96 all 360 V150116**'. Press [Insert IDs] button, follow the instructions given in the software and start priming by pressing [OK] button. If functions enabling the ID data input are deactivated, start priming directly by pressing [Start] button. 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.
4. Make sure that the samples are homogenous at the time pipetting the samples on a plate by mixing gently.

Protocol Steps

Magnetic Beads B (low well plate on plate position 2 in chemagic 360-D instrument) is resuspended by mixing thoroughly and pipetted manually (150 µL / well) to each corresponding sample well in use.

Elution Buffer (deep well plate on plate position 8 in chemagic 360-D instrument) is pipeted manually (100 µL / well) to each corresponding sample well in use.

The sample preparation steps are done manually. After the sample preparation steps, the whole blood samples can be run on one extraction plate in same automated extraction run. Upon completion of sample preparation steps (refer to sample preparation step table), the sample plate is placed to the chemagic 360-D instrument and the automated DNA extraction process is initiated.

NOTE: The automated extraction run must be started immediately after adding the Binding Buffer B to the lysed sample wells. Hesitation may result in low yield and purity.

Please refer to the table on the next page for more detailed information on plate position and protocol step details.

Automated DNA Extraction run in chemagic 360-D instrument

Position on tracking system*	Material in position	Protocol step in detail
		Select the protocol ' check manifolds H96 all 360 V150116 ' to flush the tubing prior to starting the automated extraction run. Press [Insert IDs] button, follow the instructions given in the software and start flushing by pressing [OK] button.
		When using the functions enabling the ID data input, select the protocol ' ---chemagic CS200 IVD prefilling V141203.che ' and press [Insert IDs] button. Follow the instructions given in the software to fill in the required data. Load the plates to tracking system positions 1-8. After all plates are in place, press [OK] button.
1	Rack with Disposable Tips	Use Disposable Tips according to the positions of the samples. Note: Tips need to be present in rack in full rows.
2	Low well plate 150 µL Magnetic Beads B	Pipette thoroughly resuspended Magnetic Beads B to each sample well in use. Place the plate to rack position 2.
Prepare the samples according to the procedures described in separate tables. The samples should be prepared after all the other preparing steps are ready and the plates placed on tracking system positions 1-2 and 4-8.		
3	Sample plate (Deep well plate)	Place the plate with prepared samples to rack position 3 and check all plates for accurate positioning and fitting. Close the front door and start the DNA extraction process immediately .
4	Deep well plate	Place empty plate to rack position 4. Wash Buffer BB is dispensed to the plate automatically.
5	Deep well plate	Place empty plate to rack position 5. Wash Buffer BA is dispensed to the plate automatically.
6	Deep well plate	Place empty plate to rack position 6. Wash Buffer E is dispensed to the plate automatically.
7	Deep well plate	Place empty plate to rack position 7. Wash Buffer H is dispensed to the plate automatically.
8	Deep well plate 100 µL Elution buffer	Place the prefilled Elution Buffer plate to rack position 8.
		When using the functions enabling the ID data input, the extraction run is started by pressing [Start] button at the end of the dialogue. If the functions enabling the ID data input are deactivated, load the plates to tracking system positions 1-8. After all plates are in place, select the protocol ' ---chemagic CS200 IVD prefilling V141203.che ', mark the Columns in use on the plate map in the dialogue and start the extraction run directly by pressing [Start] button.

* Numbers in tracking system refer to the positioning of the plate in chemagic 360-D instrument.

Sample preparation, Whole Blood samples

Material	Protocol step in detail
Deep well plate	
200 µL blood (sample)	Dispense up to 96 wells of the Sample Plate with 200 µL whole blood.
450 µL Lysis Buffer B	Add Lysis Buffer B to the wells containing sample and incubate the plate for 10 minutes.
1050 µL Binding Buffer B	Add the Binding Buffer B to each lysed whole blood sample well. Place the sample plate to rack position 3 and start the run immediately.

After the isolation procedure has finished, collect the DNA eluates and use the [Turn Table] button to unload the tracking system. Each click on the [Turn Table] moves the tracking system (table) clockwise by one position. Never move the tracking system (table) manually. Note: Do not turn the x-axis by hand, because it may cause damage to the equipment. All movements have to be performed with the [Turn Table] function.

PROCEDURAL NOTES

1. A thorough understanding of this package insert and the chemagic 360-D instrument manual is necessary for successful use of the chemagic DNA Blood200 kit.
2. Do not use kit reagents after the expiry date printed on the kit label. Once opened, the reagents can be used for up to 60 days.
3. Any deviation from the protocol may affect the results.
4. The reagents are automatically dispensed in whole rows and therefore the tip covers 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. The 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. Select the protocol '**regular cleaning procedure 96 dispenser 360 V150116.che**' and press the [Insert IDs] or the [Start] button 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 H96 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.

7. The amount of DNA obtained can be quantified using an independent method, e.g. UV measurement.

LIMITATIONS OF THE PROCEDURE

In some cases traces of Magnetic Beads B 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 a magnetic separator (chemagic Stand 96, provided with the chemagic 360 96 Rod Head Set prod. no. CMG-370) or centrifugation is recommended, in order to separate any traces of particles. For UV measurement, traces of Magnetic Beads may cause a higher background and a separation step should be done prior to quantitation.

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

The DNA yield strongly depends on blood characteristics, e.g. low leukocyte count results in a decreased DNA yield.

PERFORMANCE CHARACTERISTICS

The performance of the chemagic DNA Blood200 kit has been established with MSM I instrument by conducting DNA extractions for whole blood specimens from healthy donors. The mean yield for each specimen was calculated and plotted against the white blood cell count. Table 1 shows the descriptive statistics of the specimen means and the DNA yields are presented in figures 1 and 2 using both EDTA and citrate sample tubes. Note: The DNA yield is also effected by the sample dilution caused by the used sample tube (the volume of preservative is different in EDTA and citrate sample tubes).

The results on the purity of the extracted DNA are presented in figure 3.

Table 1. The descriptive statistics of the specimen results

Variable	N	Missing	Median	Mean	Minimum	Maximum
Citrate Yield (ng)	41	0	5.31	5.27	3.30	7.42
EDTA Yield (ng)	41	0	5.89	6.04	4.14	8.23

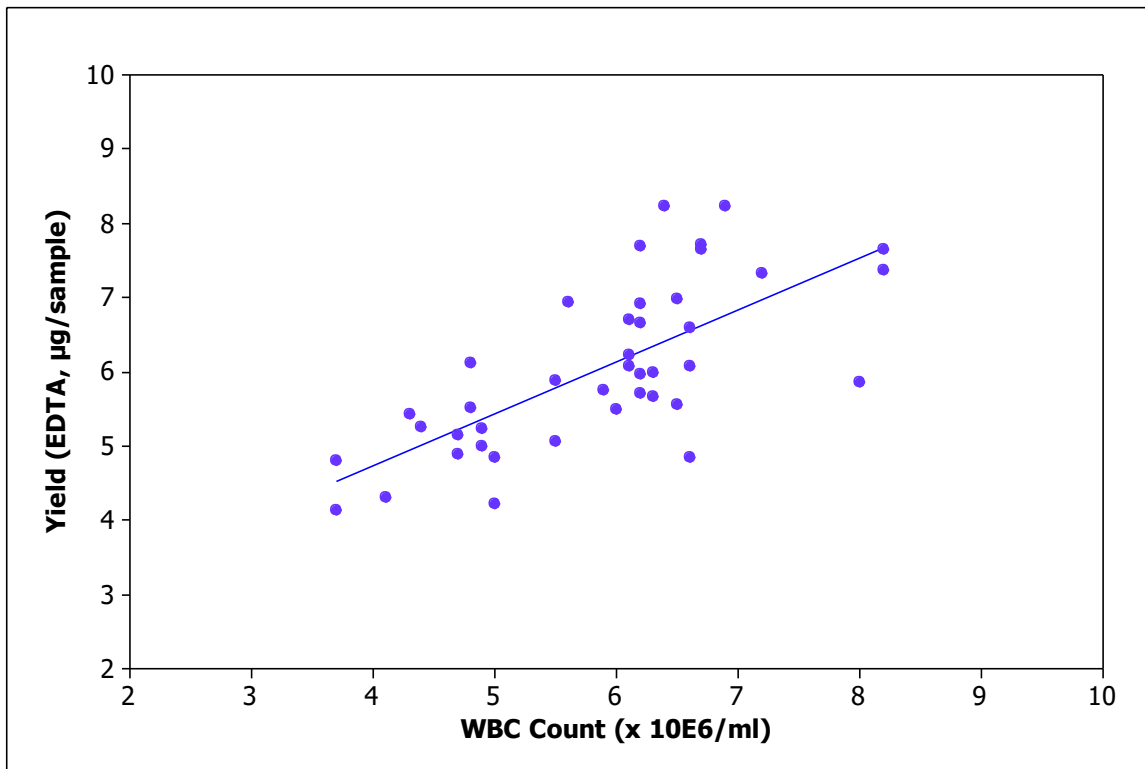


Figure 1. The DNA yield (EDTA tubes, 41 specimens) from 200 µL sample volume. White blood cell counts of healthy donors were determined and were in the range 2.9 - 8.2 x 10⁶ cells/mL.

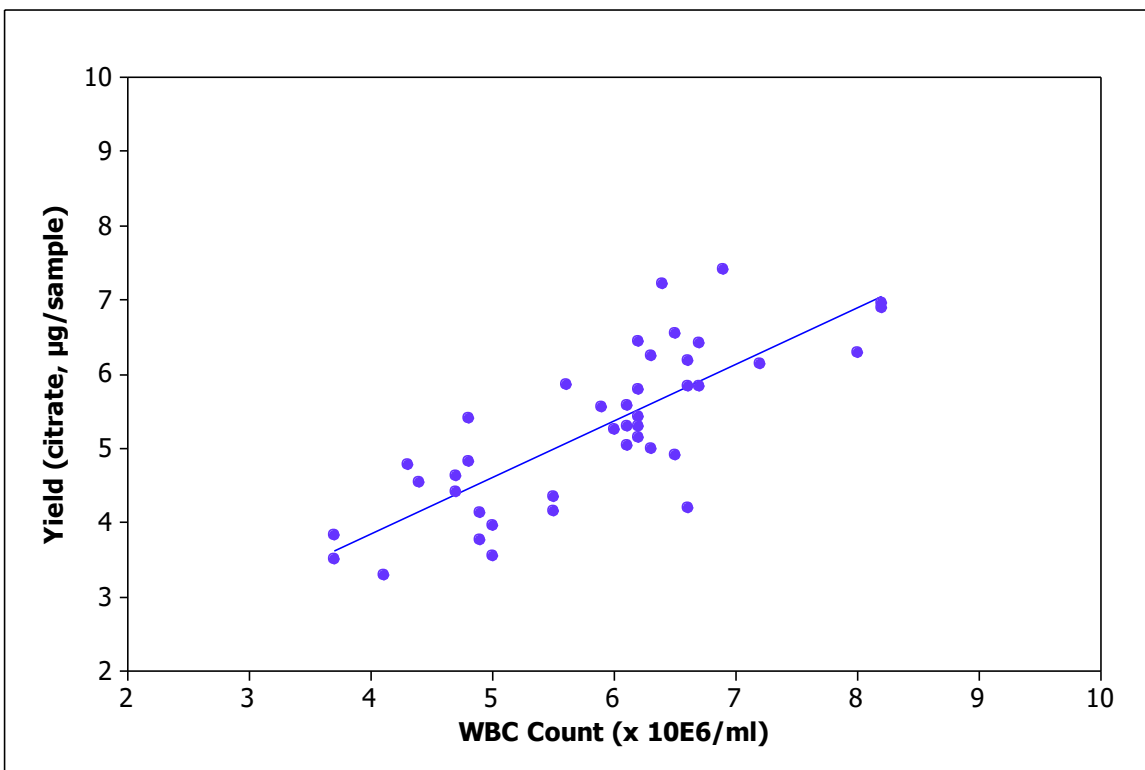


Figure 2. The DNA yield (citrate tubes, 41 specimens) from 200 µL sample volume. White blood cell counts of healthy donors were determined and were in the range 2.9 - 8.2 x 10⁶ cells/mL.

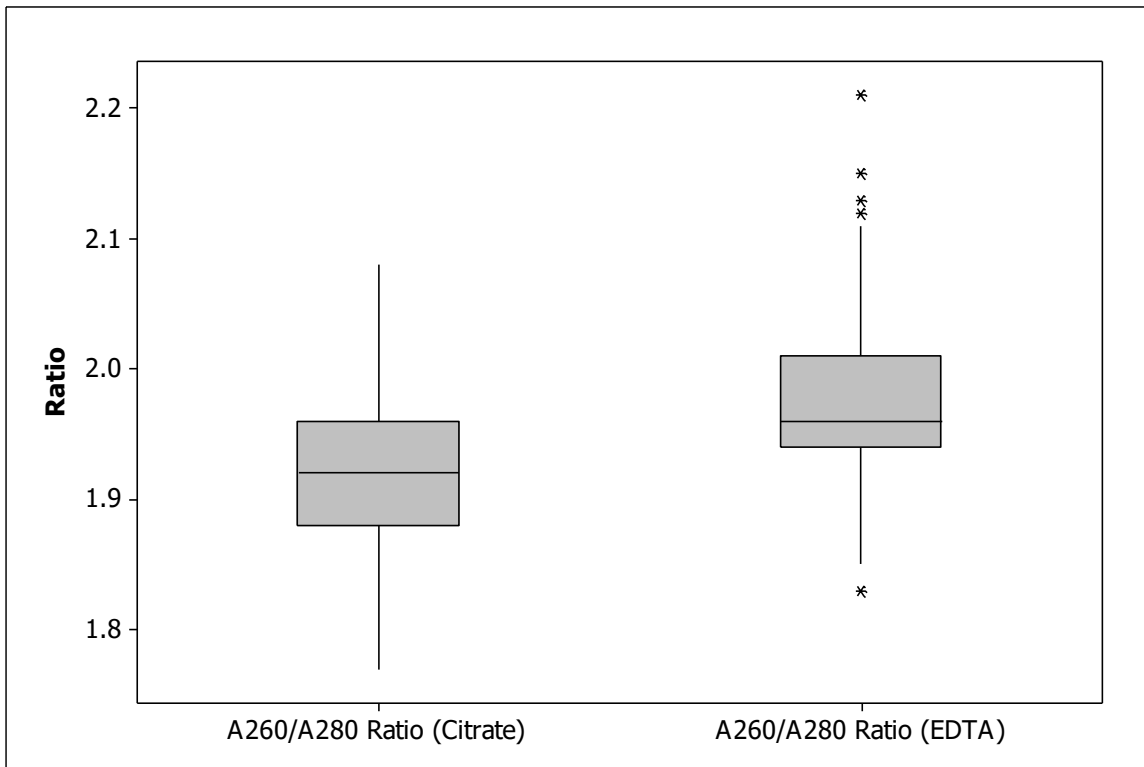


Figure 3. The purity of the extracted DNA (Absorbance ratio A260/A280) of 41 Citrate specimens (123 replicates) and 41 EDTA specimens (122 replicates).

The performance of the 3095-0030 chemagic DNA Blood200 kit with 360-D instrument was established by a comparative study with both MSM I and 360-D instruments by conducting DNA extractions for whole blood specimens from healthy donors. Five blood samples were extracted in five runs. Each sample was extracted in each run in five replicates. The DNA yields are presented in figure 4 using citrate sample tubes.

The results on the purity of the extracted DNA are presented in figure 5.

According to the results of the comparative study the yield and purity of the DNA extracted by MSM I and 360-D are similar. Therefore the DNA extraction may be performed with whichever of the two instruments.

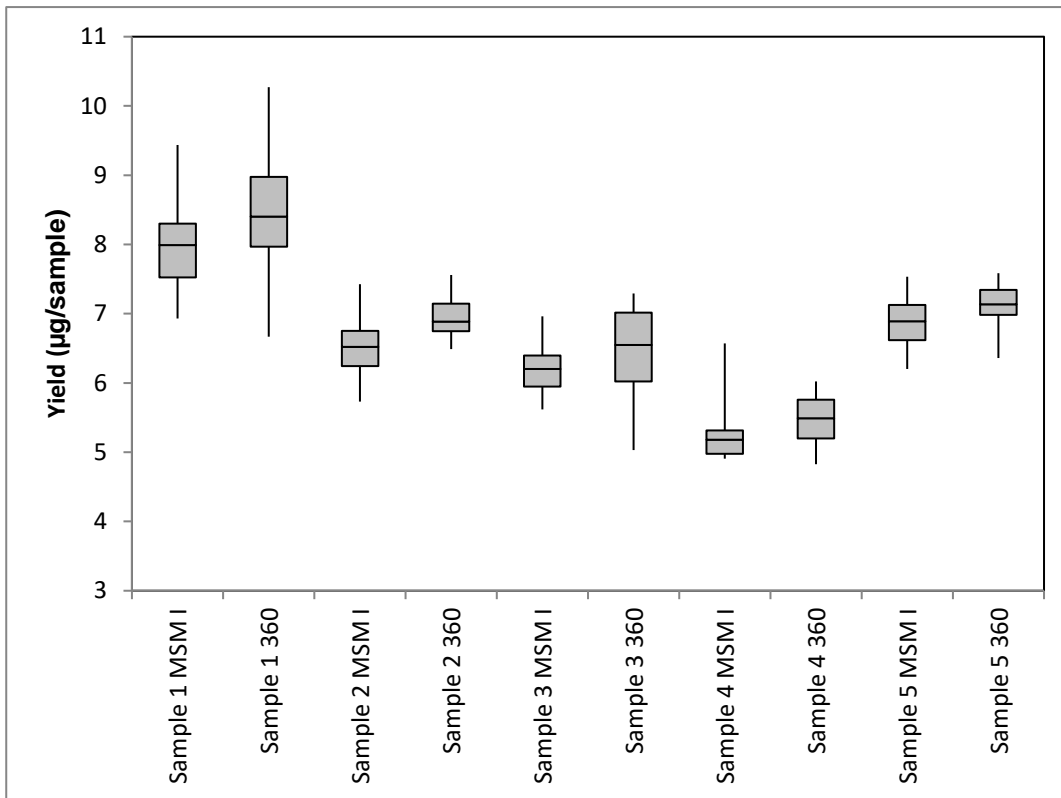


Figure 4. The DNA yield from 5 samples extracted both with MSM I and 360-D instruments.

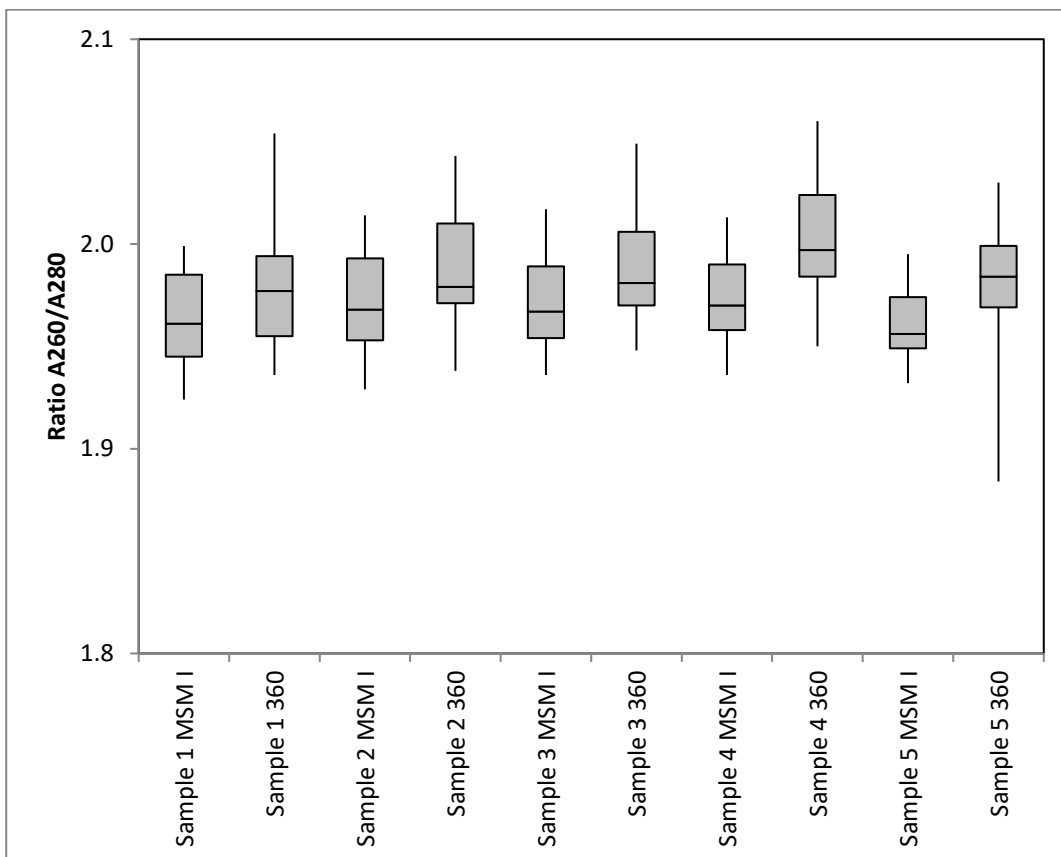


Figure 5. The purity of the extracted DNA (Absorbance ratio A260/A280) of 5 samples extracted both with MSM I and 360-D instruments.

WARRANTY

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

Wallac Oy, its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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