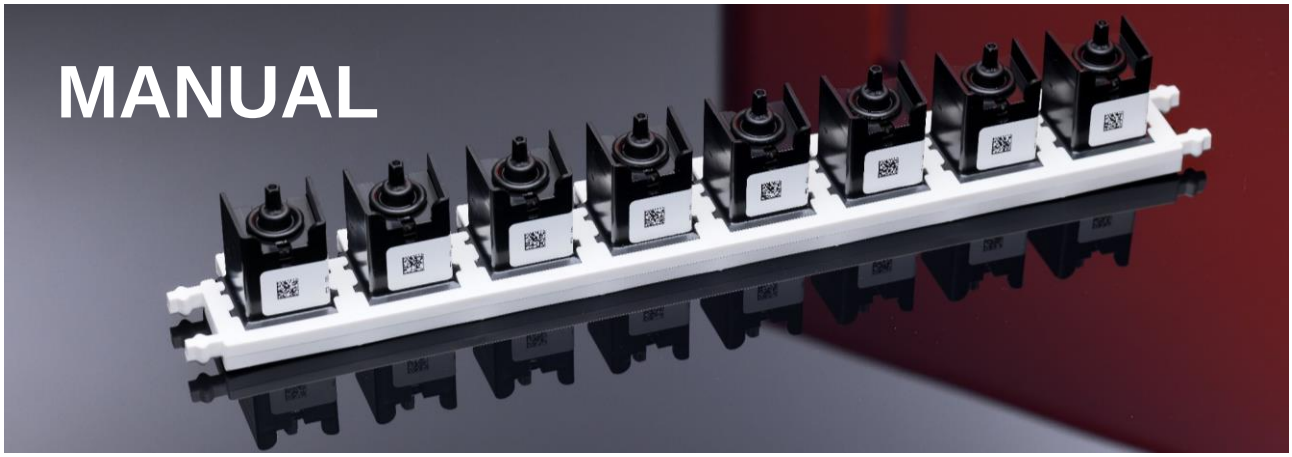
MANUAL



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IVD CE

January, 2020.

IPC

REF *HC0471-25*

GINA 500

REF *HC0400-50*

GINA 500 + DNA Purification

REF *HC0404-50*

Kit for enriching bacterial and fungal DNA from human blood or other human sample types (including DNA purification) including an Internal Process Control (IPC)

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List of abbreviations

°C ..... *Celsius degree*
µL ..... *Microliter*
CE ..... *Conformité Européenne (European Conformity)*
DNA ..... *Desoxyribonucleic acid*
DNase ..... *Deoxyribonuclease*
Dx ..... *Diagnostics*
EDTA ..... *Ethylenediaminetetraacetic acid*
g ..... *Gravity*
LOD ..... *Limit of Detection*
M ..... *Molar*
min. ..... *Minute*
Min ..... *Minimum*
mL ..... *Millilitre*
pg ..... *Picogram*
(q)PCR ..... *quantitative Polymerase Chain Reaction*
RT ..... *Room Temperature*
s ..... *Second*
UV ..... *Ultraviolet*

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Explanation of symbols

Symbol	Explanation
	CE mark. In vitro diagnostic medical device.
	Manufacturer.
	Lot/batch number.
	Catalogue number.
	Serial number.
	Keep away from rain / humidity.
	Keep away from sunlight.
	Only use once. Do not re-use.
	Don’t use if package is damaged.
	Do not eat or drink.
	Use by date.
	Temperature limit for storage.
	Sufficient for <n> tests.
R 22	Harmful if swallowed.
S 1/2	Store in a secure location and away from children.
S 18	Open and handle container with caution.
S 20	Do not eat or drink while handling.
S 24/25	Prevent contact with eyes and skin.
S 36/37	Wear appropriate protective gloves and clothing while handling.

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Introduction and intended use

GINA pathogen enrichment (and DNA purification) kits remove the vast majority of human (blood) cells and cellular debris from human whole blood and other human samples. The procedure is intended to drastically increase the percentage of pathogenic (bacterial and fungal) DNA of intact pathogens relative to human DNA in the resulting solution and to provide better conditions for downstream PCR reactions.

Quality assurance concepts for such highly sensitive molecular pathogen identification from human samples must ensure that negative results are only caused by negative samples - and not by any flaws during processing the sample. Therefore a stringent process control has to undergo the same procedures as the sample itself – without setting-off sensitivities of the tests. Cube Dx' Internal Process Control (IPC) consists of frozen biological material, which is dissolved within the human sample before the enrichment process starts. IPC undergoes the same extraction procedures as the sample itself. The follow-up PCR and hybcell test confirm the presence of IPC DNA and therefore the validity of results.

The test must be carried out in an environment suitable for molecular biological testing. This includes DNA- and DNase-free pipets, separated rooms for DNA isolation and amplification/detection and the possibility of UV decontamination. ***The test should exclusively be performed by qualified personnel, which has been trained in the use of Cube Dx' products for the identification of pathogens.***

For processing GINA kits a table-top centrifuge with a rotor for 2mL tubes, capable of applying 11.000g (e.g. Eppendorf, Hermle, etc.) and a conventional heating block (e.g. Analytic Jena, Coyote Bioscience) capable to heat up to 100°C are needed.

The kit is not intended for follow-up quantitative determination of pathogens (in terms of colony forming units) present in the sample.

Technical description

The course of sepsis or other severe infections and especially the chances of recovery and survival are dependent on early identification of the causing pathogen(s).

The chances of survival and recovery after suffering from sepsis and other severe infections are higher after an early identification and targeted treatment of the causing pathogen(s).

Cube Dx' Internal Process Control (IPC) consists of frozen biological material, which is dissolved in the sample before the enrichment procedure. This biological material is similar to pathogenic microorganisms causing sepsis or other severe infections.

The kits *GINA 500 (for 500µl of sample liquid, with or without DNA purification) a*re designed for clinical routine application to enrich pathogenic (bacterial, fungal) DNA. After enrichment the solution is purified and the eluate is for example used in PCR reactions (e.g. bacterial DNA, fungal DNA, resistance marker genes). In case that PCR products have been amplified in a sample, the respective pathogen can be identified straight-forward by Cube Dx' *compact sequencing*.

The kit is based on the following process steps:

- Lysis and removal of human cells: LE solution is added to the sample and the majority of human (and compromised pathogen) cells are lysed and removed after centrifugation.
- Lysis of pathogen cells: NA solution is added and incubated. Pelleted pathogen cells are lysed.
- Neutralization: The lysate is transferred into the T solution to stop the process of lysis and to neutralize the resulting solution.

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- *Including DNA purification*: spin column technology is used to purify DNA from the GINA solution.

It is possible that outcome is corrupted by the nature of the sample or errors during the procedure (low amount of DNA, contamination with environmental microorganisms/DNA), other influences (degraded DNA, contamination with chemicals) or technical errors .

Following circumstances deteriorate results for a sample:

- Time between drawing the (blood) sample and start of sample preparation is more than 4 hours.
- Storage of sample between drawing and start of sample preparation is not according to specification (specified: store dry and between 4°C and 8°C).

Product components

Internal Process Control (IPC):

- *IPC* (order number HC0471-25): store at **-15 to -25 °C**
  - 25 x 20µL IPC
    (25 x separately packed 0,5mL micro tubes with biological material (IPC, each 20µL))

To enrich pathogens (bacteria and fungi) from 500µl (or less) sample, following specific products are required:

- *GINA 500* (order number HC0400-50): store at **room temperature (8 to 25 °C)**
  - 2 x 25 *LE solution* (1400µl); (2 x 25 x 2mL tubes with yellow cap)
  - 1 x 12mL *NA solution* (red mark on bottle and cap)
  - 1 x 25mL *T solution* (green mark on bottle and cap)

To enrich pathogens (bacteria and fungi) from 500µl (or less) sample, following specific products are required:

- *GINA 500* + DNA Purification (order number HC0404-50): store at **room temperature (8 to 25 °C)**
  - 2 x 25 *LE solution* (1400µl); (2 x 25 x 2mL tubes with yellow cap)
  - 1 x 12mL *NA solution* (red mark on bottle and cap)
  - 1 x 25mL *T solution* (green mark on bottle and cap)
  - 1 x 30mL *Wash Buffer BW* (bottle)
  - 1 x 60mL *Wash Buffer B5* (bottle)
  - 1 x 13mL *Elution Buffer BE* (bottle)
  - 50 x *Column*
  - 50 x *Collection Tube*
  - 50 x *Elution Tube*

Pay attention not to mix up components of different lots!

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Storage and shelf life

The minimum shelf life of the products is only guaranteed, if the required temperature and humidity conditions are safeguarded during transportation and storage. The expiry dates of the products are shown on the products' labels.

IPC is delivered frozen and must be stored at **-15 to -25 °C**.

GINA 500 and *GINA 500 + DNA Purification* are delivered at room temperature and must be stored at **room temperature (8 to 25°C)**.

If the packaging (e.g. any tubes) are damaged / or the minimum shelf life has expired, the product / component must not be used. Thawing and freezing again destroys IPC and is strictly forbidden. IPC has to be used immediately after opening the tube.

Required equipment

The following equipment is required for conducting the enrichment and purification reaction:

Required Accessories / Infrastructure (or equivalents)		**REF**
Mini Vortex Mixer	Fisher Scientific[1]	
DNA work bench	Starlab[2]: Laminar Flow PCR work bench with UV-light PEQLAB[3]: PCR-working station	N2530-8200 90-UV/PCR2
Pipettes: ▪ 20 – 200µL ▪ 100 – 1000µl	GILSON[4]: PIPETMAN P200N PIPETMAN P1000N	 F144565 F144566
Sterile filter tips DNA-free: ▪ 200/300µl ▪ 1000µl	VWR[5]: 300µl Biotix 1250µl Biotix	 M-0300-9FC M-1250-9FC96
Standard table centrifuge (with rotor for 2 mL tubes)	Eppendorf[6]: Centrifuge 5430	
Standard heating block	Coyote Bioscience[7] H2O3-H	

Required accessories.

1 https://www.fishersci.com/shop/products/variable-speed-mini-vortex-mix/14955163
2 http://www.starlab.de
3 http://www.peqlab.de
4 http://www.gilson.com
5 http://www.vwr.com
6 http://www.eppendorf.com
7 http://www.coyotebio.com

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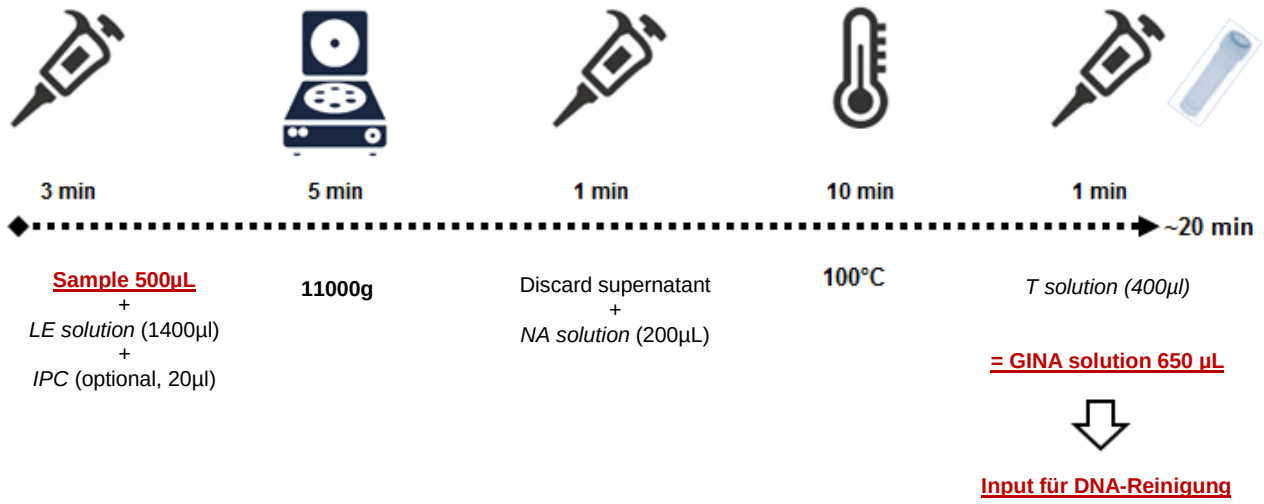
Enrichment (and Purification) procedure

The procedure starts with a native sample of EDTA-whole blood.



Note, that some steps of the procedure require the preparation of equipment or reagents. As these tasks may be associated with waiting times, read the entire chapter of the procedure before starting.

During processing the samples a laboratory coat, latex gloves, sleeve guards, hair (and beard) net and a surgical mask must be worn to avoid contamination of the test reagents. Pathogen enrichment (see steps 2.-8. below, in red) must be done under a DNA work bench.

1. Make sure that all components of the kit and the equipment are ready for use. Briefly spin down the needed tubes with *LE solution* to avoid carry-over of liquids potentially present in the screw caps, when opening the vials. Heat the heating block to 100°C.
2. Prepare *LE solution* and sample. **Do not shake or agitate the *LE solution* tube (yellow cap) to avoid the build-up of foam**! Transfer 500µl (or less) EDTA blood (or other diluted sample) into the *LE solution* (yellow cap) and pipet up and down to mix.
3. Optional: Add 20µL *IPC* to the *LE solution* / blood mixture (add *IPC* after EDTA blood!).
4. Close tube, mark it and vortex vigorously for 5 seconds or invert tubes several times. Incubate ~2 min at room temperature (18°C to 25°C).
5. Centrifuge for 5 minutes between 9000 and 11000g (preferably with 11000g). If available, use a soft ramping of the centrifugation speed.
6. Remove supernatant carefully by **decanting** and add 200µl *NA solution* (red cap) into the tube with the yellow cap. Close the screw cap tightly.
    - Remark: Some sample liquid (~50µL) may stay on top of the pellet after decanting. **Whole blood samples should turn greenish at this point.**
7. Vortex vigorously for 5 seconds. Make sure that the tubes are still tightly closed.
8. Incubate at 100°C for 10 minutes (+/- 1 minute), using a heating block.
9. Add 400µl *T solution* (green cap) into the tube with the yellow cap to neutralize.
    - Remark: **Whole blood samples should turn from greenish to dark reddish.**

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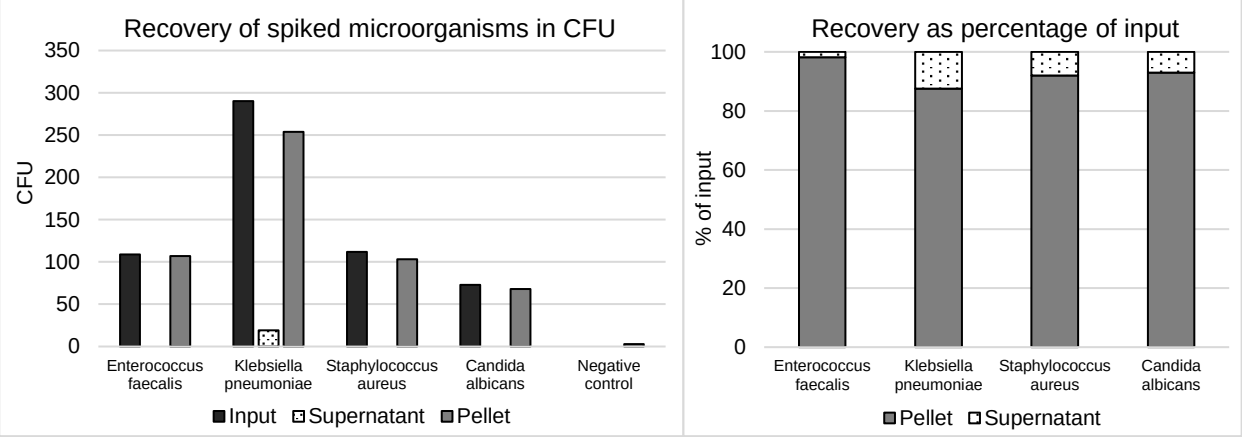
10. Purify DNA, using common DNA extraction products (in case of GINA 500 + DNA purification: Machery Nagel Nucleo Spin reagents are included in the kit. Otherwise: follow manufacturer's instructions, skip steps 11-17).
11. For each sample, place one *Column* into a *Collection Tube* and mark the *Collection Tube* with the sample ID. Transfer the whole GINA solution (600 to 650µL) to the column. Discard the tube with the yellow cap.
12. Centrifuge for 1 min between 9000 and 11000g. Remove *Column*, decant the flow-through liquid and insert *Column* again.
13. Add 500µl *Wash Buffer BW* and centrifuge for 1 minute at between 9000 and 11000g. Remove *Column*, decant the flow-through liquid and insert *Column* again.
14. Add 600µl *Wash Buffer B5* and centrifuge for 1 minute at between 9000 and 11000g. Remove *Column*, decant the flow-through liquid and insert *Column* again.
15. Centrifuge for 1 minute at between 9000 and 11000g to dry the silica membrane. Check if some liquid remains at the bottom of the *Column*. If yes, repeat this step.
16. Place the *Column* into an *Elution Tube* and mark the *Elution Tube* with the sample ID. Add 150µl *Elution Buffer BE*. Incubate at room temperature for 1min. Centrifuge for 1 minutes at between 9000g to 11000g. Check the eluation volume. If the volume appears to be too low, repeat centrifugation. Discard the *Column*.
17. Open the *Elution Tube* and incube at 100°C for 3 minutes in the heating block.
18. The collected liquid containing the DNA (eluate) might be used for PCR-based applications or stored at -20°C for later processing. Before using the eluate **vortex** the *Elution Tube* firmly.

Performance data

Recovery of pathogens: Living microorganisms (*Staphylococcus aureus Candida albicans*, *Enterococcus faecalis, Klebsiella pneumoniae*) were spiked into EDTA whole blood samples of healthy volunteers. These samples were homogenized (vortexed). Empty growth medium was spiked as a negative control. The first step of the GINA 500 protocol was executed (*LE solution* + centrifugation). The resulting pellets were resuspended in 100µl EDTA whole blood and plated out on LB agar. After centrifugation 100µl of the supernatant was plated out as well, to determine the number of living microorganisms, which were not bound in the pellet (= loss). Colonies were counted and documented after 24 to 48 hours of incubation.



The rate of recovery lies between 88% (Klebsiella pneumoniae) and 98% (Enterococcus faecalis).

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Disposal

Patient sample containers (e.g. EDTA tubes) and LE-solution tubes (yellow cap) are potentially containing infectious material and have to be disposed according to your organisation's rules for disposal of infectious material.

All other single-use materials (tubes, pipette tips, etc.) can be disposed without special disposal procedures. The usual carefulness for potentially infectious material has to be applied anyway.

Troubleshooting

In case of problems with the product, please contact:

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