

EN INSTRUCTIONS FOR USE

HISTO TRAY Complement ABC

CE₀₁₂₃

Electronic instructions for use see www.bag-diagnostics.com

REF 7003

IVD

Description of product

HISTO TRAY Complement ABC is suitable to examine the activity of rabbit complement performing the microlymphocytotoxicity test according to German and European guidelines (DGI, EFI). The ready to use microplate is predropped with three HLA-ABC antisera in defined dilutions and additional positive and negative controls. Worksheets for evaluation are enclosed.

The testing should be done using three different cell suspensions:
2 samples with corresponding antigens
1 sample with non corresponding antigens (negative control)

Using a reference sample of rabbit complement during each investigation is recommended.

Test principle

HLA-antisera react with the correspondent membrane-bound antigens on human lymphocytes. The addition of rabbit complement results in a structural change of the cell membrane which leads to a penetration of an indicator dye. Stained lymphocytes = positive reaction. In case of missing antigen-antibody reaction or a too weak complement activity, the cell membrane is intact. No penetration of indicator dye takes place and the cells remain unstained = negative reaction.

Isolation of lymphocytes

Isolation of lymphocytes from heparinized blood with a cell separation medium, e.g. HISTOPREP, for the NIH-technique or with Immuno Beads for the Immuno Beads method should be performed in accordance with the instructions for use of the manufacturer.

Rabbit Complement

Reconstitute lyophilized complement shortly before use according to instructions of the manufacturer.

Thaw frozen rabbit complement shortly before use.

Store reconstituted or thawed complement cool (2...8°C) and use it within 3 - 4 hours.

DO NOT FREEZE OR FREEZE AGAIN dissolved or thawed rabbit complement!

Test procedure NIH technique

1. Thaw plates 10 - 15 minutes before use.
2. Add 1 µl of lymphocyte suspension (2000 - 3000 cells/µl) to each well of the HISTO TRAY Complement ABC plate.
3. Incubate at 18...22°C for 30 minutes.
4. In order to examine a specific lot of rabbit complement prepare dilutions 1:2 and 1:4 with RPMI 1640 or isotonic saline.
5. Add 5 - 6 µl rabbit complement in the following manner:
 - row 3, 7, 11 dilution 1:4
 - row 2, 6, 10 dilution 1:2
 - row 1, 4, 5, 8, 9, 12 undiluted 1:1In order to avoid carry over, start pipetting the rabbit complement with low up to high concentration.
6. Incubate at 18...22°C for 60 minutes.
7. Add 3 - 4 µl eosin (5%), after 5 minutes add 5 - 6 µl formaldehyde (37%, pH 7.2) for fixation.
8. If necessary, place a coverglass onto the plate before interpretation under the microscope.
9. Allow sedimentation of lymphocytes before reading (60 minutes).

Test procedure IMB-method

1. Thaw plates 10 - 15 minutes before use.
2. Add 1 µl of lymphocyte suspension (approx. 1000 cells/µl) to each well of the HISTO TRAY Complement ABC plate.
3. Incubate at 18...22°C for 30 minutes.
4. In order to examine a specific lot of rabbit complement prepare dilutions 1:2 and 1:4 with RPMI 1640 or isotonic saline.
5. Add 5 - 6 µl rabbit complement in the following manner:
 - row 3, 7, 11 dilution 1:4
 - row 2, 6, 10 dilution 1:2
 - row 1, 4, 5, 8, 9, 12 undiluted 1:1

In order to avoid carry over, start pipetting the rabbit complement with low up to high concentration.
6. Incubate at 18...22°C for 60 minutes.
7. Add 3 µl acridin orange/ethidium bromide staining solution, 1 µl black ink and 3 µl EDTA solution (5%).
8. Use fluorescence microscope for immediate reading.

Evaluation of results

The amount of lysed lymphocytes compared with the total amount of lymphocytes is quoted as a score value in each well.

<u>% lysed cells</u>	<u>evaluation</u>
0 – 19% = Score 1	negative
20 – 39% = Score 2	doubtful negative
40 – 59% = Score 4	weak positive
60 – 79% = Score 6	positive
80 – 100% = Score 8	strong positive
= Score 0	no evaluation possible

The antigens should give clear cut reactions with corresponding undiluted antisera using minimum dilution 1:2 of rabbit complement.

Prozone phenomenons may appear depending on complement activity.

Troubleshooting**Causes of false negative or weak reactions**

- Erythrocyte contamination can make microscopic evaluation difficult
- Platelet contamination
- The amount of lymphocytes is too high
- Yellow colour of the HLA antisera
- Trays have been thawed and refrozen
- Reconstituted complement kept too long at room temperature before use
- Residual complement was frozen and thawed again.
- Incubation time were too short
- Incubation temperature were too low

Causes of false positive reactions

- Incubation time were too long
- Incubation temperature were too high
- Prior damage of lymphocytes (negative control is positive = „background“)
- Failure to add fixative

Performance Characteristics

For quality control, HISTO TRAY Complement ABC is tested with at least 4 batches of rabbit complement, wherein the undiluted antisera react unambiguously positively with the corresponding antigens at least at a complement dilution of 1:2.

Literature

Bodmer, J. et al., 1997. Tissue Antigens 49:297-321

Warnings and Precautions

HISTO TRAY Complement ABC is designed for in vitro diagnostic use only and should be applied by properly trained personnel, experienced in histocompatibility testing. Transplantation guidelines as well as EFI standard should be followed.

Human source material used to produce this reagent has been tested and found negative for HBsAg and HIV and HCV antibodies. Nevertheless all used biological material should be handled as potentially infectious, because no test method can guarantee that material derived from biological sources are free from infectious agents. When handling biological material appropriate safety precautions are recommended (Do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

Anti-HLA sera contain as preservative < 0.1% NaN₃. A concentration of < 0.1% NaN₃ is not considered to be a harmful concentration. Nevertheless avoid contact with the skin and mucous membranes. The copper and lead used in some plumbing systems can react with azides to form explosive salts. The quantities of azide used in this reagent are small; nevertheless when disposing of azide-containing materials, they should be flushed away with a large volume of water.

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

The Material Safety Data Sheet (MSDS) is available to download at www.bag-diagnostics.com.

For Formaldehyde solution and Acridinorange/Ethidiumbromide (AO/EB) please note the warnings and precautions of the manufacturers.






A yellow colouration of anti-HLA sera which still remains after thawing, may indicate a change of the pH value. Those plates should **not** be used for the test.

Do not use HISTO TRAY Complement ABC beyond the indicated expiration date on the label.

Preservative: < 0.1% NaN₃

Storage: ≤ - 20°C

Shelf life: until the expiration date indicated on the labels

Explanation of symbols used on Labelling	
	Storage temperature / Lower limit of temperature
	Use by
	Manufacturer
	Sufficient for n tests
	Consult Instructions for use
ANTI-HLA-SERA	Anti-HLA-Sera
COMPLEMENT TESTING	Intended purpose: Complement testing
CONT	Content, contains
CONTROL +	Positive control
CONTROL -	Negative control
HUM	Origin: human
IFU	Instructions for use
IVD	For in vitro diagnostic use
LOT	Batch code
MICROTESTTRAY	Microtest tray with predropped antisera and controls
MONOCL	Monoclonal
POLYCL HUM	Polyclonal
REF	Catalogue number
WORKSHEET	Worksheet

Instructions for use in other languages see:

<http://www.bag-diagnostics.com>

or phone +49 (0) 6404-925-125

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