

INSTRUCTIONS FOR USE

HISTO TRAY Complement ABC

CE0123

FOR IN VITRO DIAGNOSTIC USE

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 prefilled 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.
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.
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 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 phenomenon 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

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 specimen and test materials should be in accordance with state and local law.

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
Package: according to information indicated on the kit

Explanation of symbols used on Labelling	
	For in vitro diagnostic use
	Batch code
	Catalogue number
	Storage temperature
	Use by
	Consult Instructions for use

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