

## INSTRUCTIONS FOR USE

# HISTO TRAY HLA Class II

CE0123

## FOR IN VITRO DIAGNOSTIC USE

### Description of product

The intended use of **HISTO TRAY** is the tissue typing of HLA class II antigens using microplates with predropped anti-HLA sera and controls. Rabbit complement, worksheets for evaluation and the listing of test results are enclosed.

### 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, the cell membrane is intact. No penetration of indicator dye takes place and the cells remain unstained = negative reaction.

### Test procedure - Isolation of B-lymphocytes from e.g. heparinized blood

Isolation of the B-lymphocytes using the Immuno Beads method as well as staining and fixation reagents according to manufacturers instructions.

### Test procedure - Immuno Beads (IMB) method

1. Isolate cells with IMB-technique.
2. Bring the HISTO TRAY plates to a temperature of 18...22°C (room temperature).
3. Place 1 µl IMB-B-lymphocyte suspension (1.000 cells) into each predropped well.  
In order to guarantee sufficient antigen-antibody reaction it is necessary that antiserum and cells touch each other.
4. Incubate at a temperature of 18...22°C (room temperature) for 30 minutes.
5. Add 5 µl rabbit complement Acridinorange/Ethidiumbromide (AO/EB) (1.000 µl rabbit complement + 20 µl AO/EB).
6. Incubate for 60 minutes at a temperature of 18...22°C (room temperature) in darkness.
7. Add 5 µl EDTA-/quenching-solution (2.000 µl quenching solution + 1.000 µl EDTA 8% aqueous).
8. Read HISTO TRAY plates under a fluorescence microscope.

### Evaluation of results

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

<b>% lysed cells</b>		<b>Evaluation</b>
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

### 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**

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

### Rabbit Complement

Dissolve lyophilized complement with 1 ml or 5 ml aqua dest. (according to package sizes). The reconstitution takes 10 - 15 minutes.

Reconstituted complement must be stored cool (2...8°C) and used within 3 - 4 hours.

**DO NOT FREEZE dissolved rabbit complement!**

### Performance characteristics

Please note the listing of test results HISTO TRAY in order to receive data for diagnostic sensitivity and specificity (R-Value).

### Literature

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

### Warnings and Precautions

**HISTO TRAY plates and rabbit complement** are 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, in the particular case of doubtful results.

Human source material used to produce these reagents has been tested and found negative for HBsAg and HIV and HCV antibodies. Nevertheless all used biological material like blood, sera and control sera 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% NaN<sub>3</sub>. A concentration of < 0.1% NaN<sub>3</sub> 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 quenching 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.

Material Safety Data Sheets available on request.

Do not use **HISTO TRAY plates and rabbit complement** beyond the indicated expiration date on the label.

- Preservative:** < 0.1% NaN<sub>3</sub>  
**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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