

EN INSTRUCTIONS FOR USE

HISTO TRAY

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

HISTO TRAY AB 120 (5)	REF	7042
HISTO TRAY ABC 72 (10)	REF	7022
HISTO TRAY ABC 120 (5)	REF	7013
HISTO TRAY ABC 144 (5)	REF	7035
HISTO TRAY B27 (10)	REF	7006
HISTO TRAY B27 forte (10)	REF	7004
HISTO TRAY B27 forte (30)	REF	7007
HISTO TRAY Disease (10)	REF	70461
HISTO TRAY Disease (30)	REF	7046

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1. Intended use

In vitro diagnostic medical devices for use by qualified personnel.

HISTO TRAY AB and ABC kits

The HISTO TRAY AB and ABC kits are used for serological tissue typing of HLA class I antigens in the complement-dependent microlymphocytotoxicity test (LCT).

HISTO TRAY B27 kits

The HISTO TRAY B27 kits are designed for the serological detection of the disease-associated HLA-B27 antigen in the complement-dependent microlymphocytotoxicity test (LCT).

The association of HLA-B27 with the clinical picture of seronegative arthritis (ankylosing spondylitis, Reiter's disease, reactive arthritis) is used to support the diagnosis of these diseases.

HISTO TRAY Disease kits

The HISTO TRAY Disease kits are used for the serological detection of the disease-associated HLA antigens B27, B51, B8 and cross-reacting antigens in the complement-dependent microlymphocytotoxicity test (LCT).

The association of these antigens with diseases is used to support the diagnosis of these diseases.

HLA-B27 is associated with seronegative arthritis (ankylosing spondylitis, Reiter's disease, reactive arthritis).

HLA-B51 is associated with Behcet's disease.

HLA-B8 is associated with various autoimmune diseases (e.g. polymyalgia rheumatic, giant cell arthritis).

2. Product Description

The HISTO TRAY kits contain microplates (microtesttrays) with pre-dropped anti-HLA sera and a positive and a negative control as well as lyophilised rabbit complement. Worksheets for evaluation and result lists are included with the kits.

Exception: The product HISTO TRAY Disease (10) does not contain rabbit complement.

3. **Test Principle**

Complement dependent microlymphocytotoxicity test (LCT)

The anti-HLA antibodies in the sera react with corresponding membrane-bound HLA antigens on human lymphocytes. If the corresponding HLA antigen is present on the lymphocytes and an antigen-antibody reaction has taken place, the addition of rabbit complement causes a structural change in the cell membrane. This allows an indicator dye to penetrate the lymphocytes and they are stained (positive reaction). If no antigen-antibody reaction takes place because the corresponding antigen is not present, the cell membrane remains intact. The cells cannot incorporate the dye (negative reaction). The test is evaluated microscopically.

4. **Material**

4.1 **Contents of the HISTO TRAY kits**

HISTO TRAY AB 120 (5), 5 tests, REF 7042

- ◆ 5 x 2 Microtesttrays The plates contain 116 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control on each plate. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 5 x 1 ml Rabbit Complement Lyophilized
- ◆ 5 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY ABC 72 (10), 10 tests, REF 7022

- ◆ 10 Microtesttrays The plates contain 70 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 5 x 1 ml Rabbit Complement Lyophilized
- ◆ 10 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY ABC 120 (5), 5 tests, REF 7013

- ◆ 5 x 2 Microtesttrays The plates contain 116 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control on each plate. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 5 x 1 ml Rabbit Complement Lyophilized
- ◆ 5 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY ABC 144 (5), 5 tests, REF 7035

- ◆ 5 x 2 Microtesttrays The plates contain 140 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control on each plate. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 5 x 1 ml Rabbit Complement Lyophilized
- ◆ 5 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY B27 (10), 10 tests, REF 7006

- ◆ 10 Microtesttrays The plates contain 1 x 10 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 10 x 1 ml Rabbit Complement Lyophilized
- ◆ 10 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY B27 forte (10), 10 tests, REF 7004

- ◆ 10 Microtesttrays The plates contain 1 x 22 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 10 x 1 ml Rabbit Complement Lyophilized
- ◆ 10 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY B27 forte (30), 30 tests, REF 7007

- ◆ 10 Microtesttrays The plates contain 3 x 22 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 5 x 1 ml Rabbit Complement Lyophilized
- ◆ 10 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY Disease (10), 10 tests, REF 70461

- ◆ 10 Microtesttrays The plates contain 1 x10 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 10 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

HISTO TRAY Disease (30), 30 tests, REF 7046

- ◆ 10 Microtesttrays The plates contain 3 x 10 anti-HLA sera (polyclonal and monoclonal) and a positive and a negative control. The arrangement and specificity of the sera are given on the worksheet.
- ◆ 5 x 1 ml Rabbit Complement Lyophilized
- ◆ 10 Worksheets
- ◆ 1 Result list The result list shows the reaction patterns of the antisera.
- ◆ 1 Instructions for use

4.2 Reagents and Equipment required but not provided

Standard rabbit complement, e.g. REF 7018 (10 x 1 ml Rabbit Complement)
Only required when using the HISTO TRAY Disease (10) kit. All other HISTO TRAY kits contain rabbit complement.

Variable pipettes (0.5 - 1000 µl) and pipette tips

Aqua dest.

Microtitre syringes with dispenser for volumes from 1-5 µl

For isolation of lymphocytes (B and T lymphocytes) with density gradient centrifugation and NIH test

Cell culture medium, e.g. RPMI 1640, REF 70126

Cell separation medium, e.g. HISTOPREP, REF 70125

Centrifuge tube (12 ml)

Centrifuge

Pasteur pipettes

Neubauer count chamber or cell counter

Eosin solution (5% aqueous)

Formaldehyde solution (37%, pH 7.2)

Cover glasses for microscopy

Inverse phase contrast microscope.

For isolation of T lymphocytes using the Immunobeads technique and testing

Immunobeads Class I (required equipment see manufacturer's instructions for use)

Acridine orange/ethidium bromide (AO/EB) solution

EDTA 8% aqueous

Quenching solution

Fluorescence microscope

5. Storage and Stability

The HISTO TRAY kits are delivered on dry ice. After delivery, store the kits immediately at $\leq -20^{\circ}\text{C}$. Store in temperature monitored devices.

The lyophilised rabbit complement can also be stored in the refrigerator ($\leq 8^{\circ}\text{C}$).

The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer kit label refers to the kit component with the shortest shelf life.

Shortly before starting the test, remove the microtesttrays from the freezer and bring them to room temperature ($18\text{...}22^{\circ}\text{C}$).

The dissolved rabbit complement must be stored cool ($2\text{...}8^{\circ}\text{C}$) and used within 3 - 4 hours. DO NOT FREEZE dissolved rabbit complement!

6. Sample material and Sample preparation

T lymphocytes or mixed lymphocyte suspensions (B and T lymphocytes) are required for the test. They can be isolated from fresh peripheral blood or blood reserves (buffy coats). Blood containing anticoagulants (heparin, ACD) should be prepared within 24 hours (maximum 48 hours). Store the blood at room temperature until processing.

6.1 Isolation of Lymphocytes with Density gradient centrifugation from e.g. heparinized Blood

1. To increase the cell yield, dilute 4 ml of heparinized (50 IU/ml) blood with 4 ml cell culture medium, e.g. RPMI 1640.
2. Pipette 4 - 5 ml cell separation medium, e.g. HISTOPREP, into a centrifuge tube (12 ml).
3. Carefully add app. 6 ml of diluted blood on the gradient with a Pasteur pipette alongside the inner edge of the tube.
4. Centrifuge for 15 minutes at $1.200 \times g$ and a temperature of $18\text{...}22^{\circ}\text{C}$ (room temperature). Let the centrifuge run out without using brake function.
5. Transfer the lymphocyte ring (interphase) with a Pasteur pipette into a new centrifuge tube.
6. For washing the lymphocytes, fill up with cell culture medium, e.g. RPMI 1640, and centrifuge at $550 \times g$ for 10 minutes; discard supernatant, resuspend sediment and fill up with cell culture medium, e.g. RPMI 1640
7. Centrifuge for 10 minutes at $230 \times g$, discard supernatant, resuspend the bottom sediment and fill it up with cell culture medium, e.g. RPMI 1640.
8. Centrifuge for 10 minutes at $110 \times g$ and discard supernatant.
9. Resuspend the sediment in cell culture medium, e.g. RPMI 1640, and adjust to a final concentration of 2000 - 3000 lymphocytes per μl (Neubauer count chamber or cell counter).

6.2 Isolation of T-Lymphocytes with the Immunobeads-Technique

Please refer to the manufacturer's instructions

- when using the Immunobeads (IMB) technique for isolation of the T-lymphocytes
- regarding the reagents needed for staining and fixation

7. Reagent Preparation

Rehydrate the lyophilised BAG rabbit complement shortly before use with 1 ml aqua dest.. The rehydration takes 10 - 15 minutes. The dissolved rabbit complement must be stored cool ($2\text{...}8^{\circ}\text{C}$) and used within 3 - 4 hours. DO NOT FREEZE dissolved rabbit complement!

Prepare rabbit complement for testing with HISTO TRAY Disease (10) from other manufacturers according to the manufacturer's instructions.

Remove the microtesttrays from the freezer shortly before performing the test and bring them to room temperature ($18\text{...}22^{\circ}\text{C}$).

8. Test Procedure

8.1 Test Procedure NIH test with Lymphocytes isolated by Density gradient centrifugation

- Bring the HISTO TRAY microtesttrays to a temperature of 18...22°C (room temperature) directly before performing the test.
- Add 1 µl lymphocyte suspension (2.000 - 3.000 cells) into each predropped well.
To ensure a sufficient antigen-antibody reaction it is necessary that antiserum and cells touch each other.
- Incubate at a temperature of 18...22°C (room temperature) for 30 minutes.
- Add 5 - 6 µl rabbit complement.
- Incubate at a temperature of 18...22°C (room temperature) for 60 minutes.
- Add 3 - 4 µl Eosin solution (5% aqueous) (soft touch method) and incubate for 5 - 10 minutes.
- Fix with 5 - 6 µl Formaldehyde solution (37%, pH 7.2) (soft touch method). Allow sedimentation of cells at least 60 minutes.
- Shortly before microscopy, place a cover glass on the microtesttray for better evaluation. Evaluate with an inverse phase contrast microscope.

8.2 Test Procedure with Lymphocytes isolated with the Immunobeads (IMB) Technique

- Bring the HISTO TRAY microtesttrays to a temperature of 18...22°C (room temperature) directly before performing the test.
- Add 1 µl IMB-T-lymphocyte suspension (app.1.000 cells) into each predropped well.
To ensure a sufficient antigen-antibody reaction it is necessary that antiserum and cells touch each other.
- Incubate at a temperature of 18...22°C (room temperature) for 30 minutes.
- Add 5 µl rabbit complement acridine orange/ethidium bromide (AO/EB) (1.000 µl rabbit complement + 20 µl AO/EB).
- Incubate for 60 minutes at a temperature of 18...22°C (room temperature) in darkness.
- Add 5 µl EDTA / quenching solution (2.000 µl quenching solution + 1.000 µl EDTA 8% aqueous).
- Read the HISTO TRAY microtesttrays under a fluorescence microscope.

9. Evaluation of Results

Evaluation of the reactions: The number of lysed lymphocytes compared with the total number of lymphocytes is quoted as a score value in each well.

% lysed cells	Evaluation	
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	evaluation not possible

If the positive control does not react positively or the negative control shows a positive reaction, the test result cannot be evaluated.

10. Important Notes / Limitations of Method

The HISTO TRAY kits are for in vitro diagnostic use only.

The HISTO TRAY AB and ABC kits should only be used by trained personnel experienced in histocompatibility testing. A serological typing result without further molecular genetic testing as the sole basis for transplantation decisions is not allowed. Transplantation guidelines and EFI / DGI standards or other national laws and guidelines in the respective country must be observed, especially in case of doubtful typing results.

The HISTO TRAY B27 and Disease kits should only be used by trained personnel. The detection of the disease-associated HLA antigen indicates a genetic predisposition to the disease. This results in a higher relative risk for the corresponding disease, but a genetically predisposed carrier of the HLA antigen does not necessarily suffer from the illness. If such a disease is suspected, the detection of the disease-associated HLA markers therefore only serves to support the diagnosis. The detection of disease-associated HLA antigens must not be used as the sole basis for the diagnosis of the disease.

Cross-reactions can occur in the serological detection of HLA antigens for both tissue typing and disease association. Known cross-reactions are indicated in the evaluation documents enclosed with each kit.

Carry over effects may occur in the serological detection of HLA antigens for both tissue typing and disease association. The evaluation documents enclosed with each kit refer to the possible carry over effect of certain antisera.

The test result is invalid if the positive control does not react positively or the negative control shows a positive reaction.

A yellow colouration of the anti-HLA sera, which still remains after thawing, indicates a change in the pH value. Such microtesttrays must not be used for testing (tissue typing, detection of disease-associated HLA antigens).

Do not use HISTO TRAY microtesttrays and rabbit complement after the expiry date indicated on the label.

Causes of false reactions

Causes of false negative or weak reactions

- Erythrocyte contamination can make microscopic evaluation difficult
- Platelet contamination
- The number of lymphocytes is too high
- Yellow colour of the HLA antisera
- Microtesttrays have been thawed and refrozen
- Reconstituted complement kept too long at room temperature before use
- Residues of dissolved complement frozen and reused
- Incubation times were too short
- Incubation temperatures were too low

Causes of false positive reactions

- Cross reactions
- Incubation times too long
- Incubation temperatures too high
- Pre-damaged lymphocytes (negative control is positive = „background“)
- Failure to add fixative

11. Performance Characteristics

Performance studies were carried out with 106 blood samples (46 heparin and 60 citrate blood samples). An NIH test was performed with 46 samples and 60 samples were tested using the IMB method. The typing results obtained were compared with the results of other commercial serological HLA typing plates. The comparison showed a 99.1% agreement.

The reaction patterns of the antisera used can be found in the HISTO TRAY results list.

12. Warnings and Precautions

Human source material for the production of the test reagents has been tested by serological or molecular genetic methods for HIV, HBV and HCV. Only negative material was used for production. Nevertheless, all materials of biological origin used for the test should be considered 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 protective 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 NaN_3 as a preservative. The reagents contain < 0.1% NaN_3 which is not considered to be harmful to health. 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 contained in the reagents are small; nevertheless, when disposing azide-containing material, it should be flushed away with a large volume of water.

Disposal of all samples, unused reagents and waste must 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 the quenching solution, formaldehyde solution and Acridine orange / Ethidium bromide (AO/EB) please adhere to the warnings and precautions of the manufacturer.

13. Explanation of Symbols used on Labelling

Explanation of Symbols used on Labelling	
	Use by
	Storage temperature / Lower limit of temperature
	Storage temperature / Upper limit of temperature
	Consult Instructions for use
	Sufficient for n tests
	Manufacturer
ANTI-HLA-SERA	Anti-HLA-Sera
COMPLEMENT RAB	Rabbit complement
CONT	Content, contains
CONTROL -	Negative Control
CONTROL +	Positive Control
HLA TYPING	Intended purpose: HLA-typing
HUM	Origin: human
IFU	Instructions for use
IVD	For in vitro diagnostic use
LOT	Batch code
LYOPH	lyophilised
MICROTESTTRAY	Microtesttray with predropped antisera and controls
MONOCL	monoclonal
POLYCL	polyclonal
REF	Catalogue number
WORKSHEET	Worksheet

14. Literature

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