BAG HEALTH CARE

EN INSTRUCTIONS FOR USE

Anti-HLA Negative Control	**REF**	**61099**
Anti-HLA Positive Control	**REF**	**61098**
Anti-HLA Positive Control B-Cell	**REF**	**5931**
Anti-HLA Positive Control T-Cell	**REF**	**5930**

Microlymphocytotoxicity Test (NIH)

FOR IN VITRO DIAGNOSTIC USE CE

Description of product

Anti-HLA Positive Control consists of an anti-human lymphocyte globulin (IgG) from rabbit, which is pooled with polyspecific human antisera.
Anti-HLA Negative Control consists of a serum pool from different male donors of blood group AB, which shows no HLA antibody reactivity.
Anti-HLA Positive Control and Anti-HLA Negative Control are used as control reagents in the microlymphocytotoxicity test for in vitro diagnostic of human HLA- Class I and/or-Class II antigens / antibodies.
Anti-HLA Positive Control B-Cell and T-Cell are used in the microlymphocytotoxicity test to verify the purity of the separated cells.

The Anti-HLA Controls are lyophilized. Before use dissolve the lyophilized Anti-HLA control sera with the required volume of aqua dest. as stated on the label. The reconstitution takes 10 - 15 minutes.

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.

Isolation of lymphocytes

Isolation of the lymphocytes using density gradient or Immuno Beads method (IMB) according to manufacturers instructions.

Test procedure – Microlymphocytotoxicity test (Class I)

1. Fill the wells of micro test trays with approx. 5 - 10 µl mineral oil.
2. Pipet into each well 1 µl Anti-HLA serum under the oil using a microliter syringe. Add 1 µl of lymphocyte suspension (app. 2000 - 3000 cells) under the oil. In order to guarantee sufficient antigen-antibody reaction it is necessary that antiserum and cells touch each other.
3. Incubate for 30 minutes at a temperature of 18...22°C (room temperature).
4. Add 5 - 6 µl rabbit complement to each well and incubate for 60 minutes at a temperature of 18...22°C (room temperature) (cytolytic reaction).
5. Add 3 - 4 µl eosin solution (5% aqueous) to each well and fix after 5 - 10 minutes with 5 - 6 µl formaldehyde solution (37%) (staining and fixation).
6. After sedimentation of lymphocytes (30 - 60 min.) read the micro test trays using an inversed phase microscope. Cover the tray with a cover glass shortly before reading.

Test procedure - IMB technique (Class II)

1. Fill the wells of micro test trays with approx. 5 - 10 µl mineral oil.
2. Pipet into each well 1 µl Anti-HLA serum under the oil using a microliter syringe. Add 1 µl of IMB-B-lymphocyte suspension (app. 1.000 cells) under the oil. In order to guarantee sufficient antigen-antibody reaction it is necessary that antiserum and cells touch each other.
3. Incubate for 30 minutes at a temperature of 18...22°C (room temperature).
4. Add 5 µl rabbit complement. Incubate for 60 minutes at a temperature of 18...22°C (room temperature).
5. Add 5 µl acridineorange / ethidiumbromide / EDTA-solution
6. Read under a fluorescence microscope.

Performance characteristics

The Anti-HLA-Positive Control sera are tested in quality control with at least 50 T-and B-lymphocyte suspensions from different donors and react according to specifications positive (score 8).
The Anti-HLA Negative control sera are tested in quality control with at least 50 T / B lymphocyte suspensions from different donors and reacts according to specifications negative (max. 2 score).

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
- Sera have been thawed and refrozen too often
- 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
- Carry over due to pipetting

Limitation
Anti-HLA Positive Control is not suitable for use with DTT (Dithiothreitol). False negative reactions may occur, because the monoclonal antibodies from rabbit contained in the control may behave differently from human IgG antibodies when treated with DTT.

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

Warnings and Precautions
Anti-HLA-Control sera 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 typing 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).
The test reagents contain NaN_3 as a preservative. Do not inhale or swallow and avoid contact with the skin and mucous membranes (H- and P-phrases see below). The copper and lead used in some plumbing systems can react with azides to form explosive salts. The quantities of azide used in this reagents 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 and Acridineorange/Ethidiumbromide- solutions please note the warnings and precautions of the manufacturer.

H-and P-phrases (for non dissolved Anti-HLA sera)

H302	Harmful if swallowed.
H412	Harmful to aquatic life with long lasting effects.
P101	If medical advice is needed, have product container or label at hand.
P102	Keep out of reach of children.
P264	Wash hands thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P273	Avoid release to the environment.
P301+P312	IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell.
P330	Rinse mouth.
P501	Dispose of contents/container to the hazardous waste with special marking.

Material Safety Data Sheets (MSDS) are available to download at www.bag-healthcare.com .

Do not use **Anti-HLA-Control sera** beyond the indicated expiration date on the label.

Preservative: < 1% NaN_3 (lyophilized), Warning  ; < 0.1% NaN_3 (dissolved)

Shelf life:
- in lyophilized form until the expiration date indicated on the label
- in dissolved form: 3 months

Storage: 2...8°C, dissolved at least at ≤ -20°C

Package: 0.5 ml, human or monoclonal, lyophilized

Explanation of symbols used on Labelling			
	Storage temperature	LCT NIH	Lymphocytotoxicitytest according to NIH
	Use by		Consult Instructions for use
ANTI-HLA-SERA	Anti-HLA-Sera	IVD	For in vitro diagnostic use
REF	Catalogue number	LOT	Batch code
HUM	Origin: human	LYOPH	Lyophilised

Instructions for use in other languages see http://www.bag-healthcare.com or phone +49 (0)6404-925-125

Version 2/2015 / Issue: 2015-05

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