**Anti-HLA Control Reagents**

for the microlymphocytotoxicity test

**Package size**

<table>
<thead>
<tr>
<th>Kontroll-HLA pos.</th>
<th>REF 824 101</th>
<th>0,5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kontroll-HLA neg.</td>
<td>REF 824 106</td>
<td>0,5 ml</td>
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</tbody>
</table>

**Dilution**

- Kontroll-HLA pos. reacts cytotoxic with 81-100% lysed lymphocytes.
- Kontroll-HLA neg. reacts non-cytotoxic with 0-20% lysed lymphocytes.
- Kontroll-HLA pos. reacts weakly positive or negative.
- Kontroll-HLA neg. reacts doubtful negative, weak positive or negative.

**Principle of the Microlymphocytotoxicity Test**

For the determination of HLA antibodies, HLA antigens (suspension of lymphocytes) with known specificity are incubated with a patient serum in the presence of complement.

After the addition of the patient serum (determination of HLA antibodies), the lymphocytes will be lysed in the presence of the corresponding antibody and complement. This is made visible using staining substances like Eosin or Acridin orange/Ethidium bromide.

**Reagent Description**

Kontroll-HLA pos. is a lyophilized anti-human lymphocyte globulin (IgG) from rabbit which reacts cytotoxic with activated and non-activated lymphocytes. 0.095% sodium azide is added to Kontroll-HLA pos. as a preservative.

Kontroll-HLA neg. is a lyophilized reagent consisting of a serum pool from different healthy male donors of blood group AB which shows no cytotoxic reactions in the microlymphocytotoxicity test with randomly selected donor lymphocytes. 0.095% sodium azide is added to Kontroll-HLA neg. as a preservative.

**Statement of Precautions**

All materials of human origin used in this product have been tested and found to be non-reactive for HBsAg, anti-HCV and anti-HIV-1/-2.

However, all products of human origin should be considered to be potential transmitters of hepatitis, HIV or other infectious agents.

Appropriate safety measures are recommended.

This product contains natural rubber latex which may cause allergic reactions.

**Storage**

Lyophilized anti-HLA control reagents should be stored at 2...8°C. They are stable up to the date stated on their individual label.

Reconstituted anti-HLA control reagents have to be stored in aliquots at −20°C or below. Reconstituted anti-HLA control reagents are stable up to 18 months but maximal up to the date stated on the label if stored at −20°C or below.

Repeated thawing and freezing of the Kontroll-HLA pos. is to be avoided, this may cause decrease in reactivity!

**Procedure**

Anti-HLA control reagents have to be reconstituted with 0.5 ml distilled water before use.

The use of the reagents with the Bio-Rad Lymphoscreen product line is described in the instructions for use (art.no. REF 823 300 and 823 350). 2 µl control reagent is pipetted per well.

**Expected Values**

Kontroll-HLA pos. is made to control the correct usage of the microlymphocytotoxicity test. The reactions of lymphocytes with Kontroll-HLA pos. should lyse 81-100% of the lymphocytes.

**Quality Control**

Every batch of Kontroll-HLA neg. is tested with at least thirty T-lymphocyte suspensions and twenty B-lymphocyte suspensions of different blood donors.

Maximal 20% lysed lymphocytes (score 2) should be detected in each well.

Every batch of Kontroll-HLA pos. is tested with at least thirty T-lymphocyte suspensions and twenty B-lymphocyte suspensions of different blood donors.

A score 8 value (81 – 100% lysed lymphocytes) is guaranteed one titer dilution below the working solution.

**Specific Performance Characteristics**

Kontroll-HLA pos. reacts positive with human lymphocytes (score 8).

Kontroll-HLA neg. reacts non-cytotoxic with human lymphocytes (negative).

**Limitations**

1. **Kontroll-HLA pos. reacts weak positive or negative:**
   - The activity of complement is too low.
   - Unproper storage of HLA trays (e.g. with dry ice, wrong temperature, HLA trays thawed and frozen again).
   - Incubation times in the microlymphocytotoxicity test are too short.
   - Incubation temperature in the microlymphocytotoxicity test is too low.
   - Using DTT (Dithiothreitol) can cause false-negative reactions as rabbit antibodies in Kontroll-HLA pos. may react different than human IgG antibodies.

2. **Kontroll-HLA neg. reacts doubtful negative, weak positive or positive:**
   - Prior damage of the lymphocytes (storage of the lymphocyte suspension too long or not in accordance with the rules).
   - Carry over by using Hamilton syringes.
   - Incubation times in the microlymphocytotoxicity test are too long.
   - Incubation temperature in the microlymphocytotoxicity test is too high.
   - Failure to add fixative.

**References**

4. ISO 15223 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied.

**Key:** Underline = Addition or significant change ▼ = Deletion of text