

# Manual CTS TRAYS

## Reagent Description:

### CTS Serum Trays HLA-AB1, HLA-AB2, HLA-DR

CTS trays contain human, polyclonal HLA antisera or monoclonal antibodies of stated specificity for the determination of HLA class I or HLA class II antigens. All antisera are tested against a well-defined panel of HLA-typed lymphocytes.

Each well contains 1 $\mu$ l anti-HLA reagent and 5–6 $\mu$ l covering oil.

## Intended Use

CTS trays are ready-to-use microplates containing pre-loaded anti-HLA reagents. They are provided for HLA laboratories at centers participating in the CTS study. CTS trays are used for the determination of human HLA antigens in a complement-dependent microlymphocytotoxicity test.

3 different trays are used for the determination of one HLA-A,-B,-DR type:

Tray AB 1  
Tray AB 2  
Tray DR

The order and specificity of pre-loaded anti-HLA reagents can be found in the worksheet enclosed with every kit.

## Principle of the Microlymphocytotoxicity Test

For the determination of HLA antigens, HLA antibodies with known specificity must be incubated with lymphocytes in the presence of complement. Lymphocytes are lysed in the presence of the appropriate antibody and complement. This is made visible using a stain and the test result is scored using a special microscope.

## Specimen Collection and Preparation

1. Isolation of lymphocytes
2. Separation of T and B lymphocytes
3. Adjustment to a concentration of 2000–3000 lymphocytes/ $\mu$ l

## Microlymphocytotoxicity Test Procedure

Thaw CTS trays at room temperature immediately before use  
Add 1 $\mu$ l of the adjusted lymphocyte suspension to each well  
Incubate for 30 minutes  
Add 5 $\mu$ l complement to each well  
Incubate for 60 minutes  
Add 3–4  $\mu$ l 5% eosin to each well  
Fix the reaction by adding 6–7  $\mu$ l of buffered formaldehyde to each well  
Alternatively, ethidiumbromid-acredinorange and ink can be used for staining

## RESULTS:

The reactions are read under an inverse phase contrast microscope (eosin) or a fluorescent microscope (ethidiumbromid - acredinorange). The number of lysed lymphocytes compared with the total number of lymphocytes is recorded as a score value for each well.

10%	=	Score 1	negative
20%	=	Score 2	doubtful negative
50%	=	Score 4	weak positive
80%	=	Score 6	positive
100%	=	Score 8	strong positive
		Score 0	not readable

## **Specificity and Reaction Strength of HLA Reagents**

The anti-HLA sera have a defined specificity. Multispecific sera are used only if no monospecific reagents are available for a particular specificity. The reagents were selected to show positive reactions (Score 4–8) with lymphocytes carrying the relevant antigen and negative reactions (Score 1–2) with lymphocytes not carrying the relevant antigen. The HLA antigen specificities of an individual are defined by the reaction pattern of positive reactions.

### **Quality Control**

Negative control: The negative control should show 5-20% lysed lymphocytes. All reactions in the tray are scored by comparison with this negative control.

Positive control: The positive control should show 81-100% lysed lymphocytes.