

# GenTrak, Inc.

## HLA Phenotyping Trays-Class I and Class II

 **For In-Vitro diagnostic use**

 **Intended Use**

### Summary:

GenTrak HLA Class I and Class II phenotyping trays consist of plastic trays with wells containing 5 µl of mineral oil and 1 µl of known antisera. Antisera specificity was serologically determined using a well defined fresh and frozen cell panel. They are used for the detection of Class I or Class II antigens on T or B lymphocytes. Each tray has a negative and positive control. The Class II tray also has anti-T cell, anti-B cell and anti-monocyte controls.

### Principle:

Viable lymphocytes are incubated with antisera in the presence of complement. Lymphocytes possessing antigens that correspond to antibodies in the antisera are lysed. This reaction is made visible microscopically by the addition of a dye indicator such as eosin-y or a staining quench agent. Cells that exclude the dye and remain viable are negative. Cells that absorb the dye are positive and possess the antigen.



**Caution:** Some sera may contain sodium azide.

Sodium azide reacts with lead and copper plumbing resulting in possible explosive conditions. If discarded in a sink, flush the sink with large amounts of water to prevent build-up of azide. Sodium azide is also poisonous and toxic if ingested.

All blood products should be handled as if potentially infectious. Human source material from which this product was derived was found non-reactive for HbsAg, HIV and HCV by FDA approved methods. No known test method can offer absolute assurance that products derived from human blood will not transmit infectious diseases.

### Product Components:

<u>Description</u>	<u>Qty</u>	<u>Storage</u>
1. 72 well trays containing 1 µl of leukocyte typing sera in each well. The antisera is of human source or a monoclonal antibody	10/box	-65° or colder
2. Class I or Class II Rabbit Complement	5 ml bottle	-65° or colder
3. Tray recording work-sheet with a certificate of analysis	1/tray	Room Temp.
4. Direction Insert	1/box	Room Temp.

### Specimen Collection

1. Blood should be collected in sodium heparin or acid citrate dextrose (ACD) vacutainer tubes and mix well by inversion.
2. Store blood at room temperature (20-24°C)
3. Viable lymphocytes are required for serological

typing. It is recommended the blood be processed within 24-48 hours after collection. Methods for isolation and storage of isolated lymphocytes may be found in the ASHI Laboratory Procedure Manual.

4. Isolated lymphocytes should be adjusted to a concentration of 1.5 - 2.5 x 10<sup>6</sup> cells/ml.

### Materials/Reagents/Equipment

#### Provided:

1. Phenotyping trays - Class I or Class II
2. Tray Worksheets - lot specific
3. Rabbit Complement - frozen, ready to use

#### Required but not Provided:

1. Lymphocyte suspension medium: RPMI 1640, McCoy's, Barbitol Buffer or comparable medium.
2. Centrifuge
3. Light Microscope
4. Inverted phase contrast or Fluorescent microscope
5. Microliter syringes - to deliver 1 µl and 5 µl.
6. Hemacytometer - improved Neubauer .1 mm deep.
7. Glass cover slips 75 x 50 mm
8. Aqueous eosin-y - 5% w/v
9. Staining Quench
10. Formaldehyde - 37% reagent grade. Adjust pH of the formaldehyde with sodium hydroxide. Store at room temperature.
11. Temperature controlled room - maintain at 22°C ±2°.
12. Freezer capable of maintaining -65°C or colder.

### Directions for Use

**NOTE:** Isolate lymphocytes following the user laboratory preferred procedure or refer to the ASHI laboratory manual.

1. Thaw trays for 15 minutes at room temperature. Use within 30 minutes of thawing. Do not refreeze.
2. Using a microliter syringe, add 1 µl of 2 x 10<sup>6</sup> cell suspension T - lymphocytes to each well of the Class I tray and B -lymphocytes to the Class II tray. Ensure cells and sera mix.
3. Incubate for 30 minutes at room temperature (20-24°C)
4. Using a microliter syringe add 5 µl of rabbit complement to each well and incubate at room temperature for 1 hour.
5. For Dye exclusion:
  - Add 2 µl of 5% eosin y to each well
  - Incubate for 5 minutes
  - Add 5 µl of 37% formaldehyde solution to each well.
  - Allow cells to gravity settle for 90 minutes
  - Cover slip the trays with 50 x 75 mm coverslip
  - Examine each well microscopically and record the percentage of cell death - non viable cells. Non-viable cells are large and stained red. Viable cells are small, unstained and refractile.
6. For Fluorescent testing:
  - Add 5 µl of a staining quench
  - Incubate the trays in the dark at room temperature for 15 minutes.
  - Examine each test well microscopically and record the percentage of non-viable (reactive) cells. Non-viable cells are large and stained red. Viable cells are small, refractile and stained green.

## Results

### Quality Control

1. The positive control is in well 1A. The percentage of cell death in this well should be 80% or greater. This control is used to determine complement reactivity.
2. The negative control is in well 1B. The percentage of cell death in this well should be 0 - 20%. This control is used to determine the viability of the lymphocyte cell suspension.
3. Anti-B cell control - this control is in well 1C and well 1D of the Class II phenotyping trays. This antibody will cause cell death in the B cell population only and indicates the purity of a B-cell preparation.
4. Anti-T cell control - this antibody is in well 1E of the class II phenotyping tray and will cause death of T lymphocytes only. This is also an indicator of the purity of B lymphocytes in the cell preparation.
5. Anti Monocyte control - this antibody is in well 1F of the Class II phenotyping tray and will cause death of monocytes only. This is also an indicator of the purity of B-lymphocytes in the cell preparation.

### Interpretation

Results are determined by the percentage of cell death observed microscopically. Cell death will occur if a cell surface antigen is detected by an antibody in the presence of rabbit complement.

*The viability of the lymphocyte preparation is established by the negative control. After reading the negative control, the percentage of cell death in the remainder of the tray are adjusted accordingly using the percentage of cell death in the negative control as a baseline.*

Results are determined using the following grading system:

<u>% Dead Cells</u>	<u>Score</u>	<u>Interpretations</u>
1-10	1	Negative
11-20	2	Weak Negative
21-50	4	Weak Positive
51-80	6	Positive
81-100	8	Strong Positive
- -	0	Unreadable

### Limitations

Isolated cells contaminated with platelets, red cells, bacteria, monocytes or granulocytes may cause the following:

**False positive reactions can be the result of the following:**

1. Cell suspension is contaminated with granulocytes
2. Cell suspension has low viability
3. One or more reagents of the cell suspension is contaminated with bacteria.

**False negative reactions can be the result of the following:**

1. Excessive platelet contamination. Platelets have HLA A & B antigens and can deplete antibody.
2. Cell suspensions are adjusted to a concentration greater than  $1.5 - 2.5 \times 10^6$  cells/ml.
3. Monocyte contamination in DR typing. Monocytes have DR antigens and no DQ antigens.
4. Cells and complement fail to mix with the antisera.

5. Bacteria contamination in cell suspension. Bacteria have receptors for antibodies and can absorb them.
6. Complement was not properly thawed or mixed.
7. Complement was inactivated. Complement is heat labile.
8. Erythrocyte contamination can deplete complement.

### Specific Performance Characteristics

Each antisera has been characterized against a 70 member or greater frozen cell panel with viability of at least 80%. This panel also represents a diverse ethnicity.

### X. REFERENCES

1. Zachary, A.A.; Teresi, G.A: ASHI Laboratory Manual Second Edition, 1990. The American Association for Histocompatibility and Immunogenetics.
2. Tissue Typing Reference Manual, South-Eastern Organ Procurement Foundation Second Edition, 1987.
3. Manual of Tissue Typing Techniques, National Institute of Allergy and Infectious Disease, Washington, DC, U. S. Department of Commerce, 1979-1980, pp 13-14.
4. Teraski, P.I; Mcllelland, J. D: Microdroplet Assay of Human Cytotoxins, Nature 204: 998, 1964.
5. Mittal, K. K; Terasaki, P. I.: Serological Cross-reactivity in the HLA System, Tissue Antigens 4: 146, 1974.
6. Svejgaard, A.; Kissmeyer-Nielsen, F: Cross-reactive Human HLA Isoantibodies, Nature 219: 868, 1968.
7. Duquesnoy, R. J.; cited by Cohen, E.: HLA-B27 and Ankylosing Spondylitis Hyland Therapeutics Division, Travenol Laboratories, Inc., Glendale, CA 1980, p. 6.
8. Bodmer, J. G.; Marsh, S. G.; et al: Nomenclature for Factors of the HLA System, 1991. Tissue Antigens, 1992.

### XI. BIBLIOGRAPHY

- Histocompatibility Testing 1970. Copenhagen, Munksgazard, 1970, pp.1-656.  
Histocompatibility Testing 1972. Copenhagen, Munksgazard, 1972, pp.1-771.  
Histocompatibility Testing 1975. Copenhagen, Munksgazard, 1976, pp.1-1035.  
Histocompatibility Testing 1998. Los Angeles, UCLA Tissue Typing Laboratory, 1980, pp. 1-1227.  
Boyum A: Separation of Leukocytes from Blood and Bone Marrow, Scand J Clin Lab Invest 21: Suppl. 97, 1968, pp. 9-76.  
Collins, Z.V., Shepherd, L.P, Tarris, R.H. et al: Separation and Prolongation of Viability of Human Lymphocytes for Tissue Typing. Evaluation of a Technique. Transfusion 10:21. 1970.  
Momchilov, B, Genecheva, G: Studies on the Assessment and Titration of Antilymphocytic Sera In Vitro and In Vivo. 13th International Congress of IABS.  
Budapest, 1973: Develop Biol Stand 27: 229, 1974.  
The American Association for Clinical Histocompatibility Testing, New York, 1990.  
Immunobiology of HLA. Histocompatibility Testing 1987. Volume I. Springer-Verlag, 1989.  
Immunobiology of HLA, Immunogenetics and Histocompatibility. Volume II. Springer-Verlag, 1989.

**Manufactured by:**

**GenTrak, Inc.**

**PO Box 1290**

**121 W. Swannanoa Ave.**

**Liberty, NC 27298**

**Phone: 1-800-221-7407 or (336) 622-5266**

**Fax: (336) 622-1750**

REV. 3: 01-07