GenTrak, Inc.

SeraScreen
FCT 30- Two Tests/Tray
FCT 60 – One Test/Tray
Reagent Controls-Four/Tray

For use in the identification of HLA-A,B or C antibodies in sera by a complement dependent micro-lymphocytotoxicity assay.

IVD **For In Vitro Diagnostic Use**

I. **SUMMARY**

The GenTrak Frozen Cell Trays contain isolated lymphocytes derived from HLA phenotyped normal individuals in a 72-well microtiter tray. They may be used for primary screening of antisera as well as assignment of HLA-A,B or C antibody specificity detectable by the complement dependent microlymphocytotoxicity assay. Reagent complement controls are provided in the designated locations on the tray.

II. **PRINCIPLE OF PROCEDURE**

Viable human lymphocytes possess HLA antigens which may be specificity bound by anti-HLA antibodies present in the test sera. With the addition of rabbit complement, cytotoxic changes may occur and be observed microscopically after addition of eosin or fluorescent dye.

III. **REAGENT**

GenTrak Frozen Cell Trays:

- **Lymphocytes are isolated from whole blood or pheresis residues according to standard methods.**
- **Each well of the tray contains lymphocytes originally derived from a freshly drawn donor which are isolated, adjusted to a final concentration of 3.5 – 5.0 X 10^4/ML in a freezing solution of DMSO (Dimethylsulfoxide) in fetal bovine serum, added to the tray under mineral oil to retard evaporation and subsequently frozen.**
- **Store at -65° or colder.** 
- **Thaw prior to use at room temperature for 5-10 minutes. Do not refreeze.**
- **The viability of lymphocytes on the GenTrak Frozen Cell Trays may decrease during the dating period.**

CAUTION: HUMAN SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NONREACTIVE FOR HbsAg, HCV AND HIV ANTIBODY WHEN TESTED WITH LICENSED REAGENTS. THE FDA CURRENTLY RECOMMENDS THAT ALL HUMAN BLOOD DERIVED SPECIMENS AND REAGENTS BE HANDLED AT THE BIOSAFETY LEVEL 2 AS OUTLINED IN THE CENTER FOR DISEASE CONTROL/NATIONAL INSTITUTES OF HEALTH MANUAL ('BIOSAFETY IN MICROBIOLOGICAL AND BIOMEDICAL LABORATORIES," 1984.) NO KNOWN TEST METHOD CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT HEPATITIS, AIDS OR OTHER DISEASES.

For *In Vitro* Diagnostic Use.

I. **SPECIMEN COLLECTION AND PREPARATION**

A suitably prepared source of human serum is required for testing. It may be collected by drawing a sample of blood and handling as follows:

A. Draw blood according to accepted medical practice into a syringe, vacutainer or other suitable container.

B. Blood is allowed to clot and then is centrifuged at 2000 RPM for 10 minutes. If plasma is utilized, it must be converted to serum prior to testing.

C. Serum is aliquoted and diluted as appropriate. If testing is deferred, the serum should be stored at -65^{0}C or colder until assayed.

II. **PROCEDURE**

A. Material Provided:

1. **GenTrak Frozen Cell tray**
2. **GenTrak Frozen Cell tray worksheets**
3. **GenTrak Rabbit Complement – frozen**
4. **GenTrak positive and negative controls**

B. Additional Material Required

1. **Media for washing, e.g. RPMI-1640, MEM or McCoy's with 20% HIFCS**
2. **Microliter syringes**
3. **Pasteur pipettes**
4. **Inverted phase contrast or fluorescent microscope**
5. **Glass coverslides**
6. **Fetal Bovine Serum**
7. **Eosin Y or Fluorescent Dye**
8. **Formalin**
9. **Centrifuge and carriers**

C. Optional Materials

1. **SeraTrak Antibody Analysis Software system optional computer software supplied by GenTrak for the analysis of GenTrak Frozen Cell trays.**

D. Test

1. **Prepare and dilute sera to be tested as required.**
2. **Remove the GenTrak Frozen Cell Tray from the freezer and allow it to thaw at room temperature for 5-10 minutes. NO MORE THAN 15 TRAYS SHOULD BE PROCESSED IN A SINGLE BATCH. DELAYS IN PERFORMANCE OF THE TEST PROCEDURE MAY LEAD TO LOSS OF CELL VIABILITY.**
3. **Add 5 uL of media solution to each well. Add the solution directly into the bottom of the well.**
4. **Allow cells to gravity settle for 10 minutes at $22^0C \pm 2^0C$. Alternatively, centrifuge the trays(s) by bringing the centrifuge speed up to 1500 RPM and then immediately decelerating to the speed to 0 RPM. Deceleration should be performed without the centrifuge brake.**
5. **Flick out the wash solution just prior to adding serum. Moderately flick (firm,not hard) into an appropriate receptacle. NOTE: DO NOT ADD OIL TO THE WELLS PRIOR TO ADDING THE SERUM!**
6. **Add 2 uL of the serum test sample (antibody). DO NOT ADD sera to reagent control wells (Row 11). Complement, eosin and formaldehyde must be added to all wells.**
7. **Incubate for 30 minutes at $22^0C \pm 2^0C$.**
8. **Add 5 uL of GenTrak Rabbit complement to the entire tray.**
9. **Incubate for 60 minutes at $22^0C \pm 2^0C$. Complement incubations may have to be adjusted according to the sensitivity desired.**
10. **Add 5 uL of fluorescent dye to the entire tray. Read tray after 15 minutes (for fluorescence).**

Alternate Method:

11. **Add 3-5 uL aqueous eosin Y to entire tray. Incubate 3 minutes at room temperature.**
12. **Add 5-8 uL of 37% neutralized Formaldehyde to the entire tray.**
13. **Let tray settle 1 hour before reading.**

III. Worksheet

The GenTrak Frozen Cell Tray worksheets provided are keyed to lot number assignments clearly marked on each tray and sheet. CAUTION; FROZEN CELL TRAYS AND WORKSHEETS MAY ONLY BE USED TOGETHER WHEN THEY HAVE THE IDENTICAL LOT NUMBER.

IV. INTERPRETATION OF RESULTS

The trays are examined utilizing an inverted phase contrast or fluorescent microscope and the results may be scored on the appropriate worksheet as follows:

% of Non-Viable Lymphocytes	Grade	Interpretation
1-10%	1	Negative
11-20%	2	Negative
21-50%	4	Weak Positive
51-80%	6	Positive
81-100%	8	Strong Positive
Unreadable	0	Invalid

CAUTION: Extreme care must be taken in the interpretation of test results and the assignment of antigen specificities.

A. Automated Analysis with SeraTrak

Possible antibody specificities in sera may be identified by the use of GenTrak's SeraTrak Antibody Analysis Software System, an optional computer program supplied to qualifying customers. The SeraTrak system is a set of programs and databases that allow the laboratory to automatically calculate the possible antibody specificities found in sera samples from patients and reagents screened on frozen cell trays. The program allows:

- **A. The entry of panel members for defining any frozen cell tray designs.**
- **B. The entry of all sera data, including demographics and final antibody assignments.**
- **C. The entry of tray scores directly from the microscope into the computer system.**
- **D. The entry of patient data, including demographics and user validated HLA types.**
- **E. The viewing of possible antibody specificities for a serum, based upon the automatic calculation of the tabulated tray scores entered for a serum.**
- **F. The printing of reports that indicated a patient's antibody history. SeraTrak offers the user a friendly, Windows-based environment that has a built-in "Help" manual designed to speed user training and daily work flow.**

Automated Antibody Analysis

The SeraTrak system will analyze the scores entered for a frozen cell tray against the HLA antigen types of the panel cells loaded into the wells of the tray. The system skips through each HLA antigen in the HLA antigen databases and determines whether each cell in each well contains the antigen in question. The following table illustrates how the analysis routine will "grade" each antigen against each cell's HLA type and tray score:

Antigen	Cell HLA type	Score	Reaction
A1	A2,A3,B7,B44	1	TN(True Negative)
A1	A2,A3,B7,B44	6	FP(False Positive)
A1	A1,A2,B7,B44	8	TP(True Positive)
A1	A1,A2,B8,B37	1	FN(False Negative)
A3	A2,A3,B7,B44	1	FN(False Negative)
A3	A2,A3,B7,B44	6	TP(True Positive)
A3	A1,A2,B8,B37	8	FP(False Positive)
A3	A1,A2,B8,B37	1	TN(True Negative)

Each Antigen is analyzed against either all 30 or 60 cells in a 30 or 60 cell frozen tray. From the above tabulated data, a Chi-square and r-value are calculated. Chi-square values determine "goodness of fit" for a set of data and will provide a value to judge how much confidence you may have in the decision that the specificity is in fact the correct antibody. Another statistic derived from the Chi-square value is the r-value (Correlation Coefficient). The r-value is a common statistic that is used to qualify how well an antibody has performed. The following formulas are used by SeraTrak to calculate the above statistics.

$$\text{CHI-SQUARE} = \frac{((TP \times TN) - (FN \times FP))^2 \times N}{(TP+FN) \times (TP+FP) \times (FN+TN) \times (FP+TN)}$$

Where TP= True Positives, TN= True Negatives, FP= False Positives, FN = False Negatives, N= total number of cells run.

Note that N= TP + TN + FP + FN

Then the r-value is calculated:

R-VALUE = SQUARE ROOT (CHI-SQUARE/N)

The statistically significant HLA antibody specificities for a serum are provided either on screen or in print for a particular serum and frozen cell tray. This data is then used by the laboratory for the manual assignment of the final antibody specificities for the serum. Please note that SeraTrak will NOT automatically assign antibody specificities to a serum under any circumstances. Please consult the SeraTrak Users Manual or the "Help" section of your SeraTrak Antibody Analysis computer program for specific details and operating instructions for using the SeraTrak system.

V. QUALITY CONTROL

A. Positive controls must be run with each day's assay to demonstrate both adequate cell and complement reactivity.

B. Negative controls must be run with each day's assay to demonstrate each individual cell's viability, and to be used as a base line for comparison with positive reactivity for each cell. Base line viability of less than 75% indicates that care should be taken in the assignment of antibody specificity based upon that cell.

C. Reagent complement controls, two each of positive and negative reactivity are provided to assure the appropriate performance of the lymphocyte microcytotoxicity assay.

VI. LIMITATIONS

A. Each lot of GenTrak Frozen Cell Trays can confirm only reactivity of antisera corresponding to the antigens included on the cell panel. See accompanying Worksheet for antigen identifications.

B. Improper thawing or washing may cause decreased viability by cell injury.

C. The viability of the cells may decrease during the dating period.

D. Refreezing the tray once thawed will affect the viability of select cells and is not recommended.

E. HLA specificities are defined by compilation of data obtained through international exchange of antisera and leukocytes.

F. Cross-reactivity in the HLA system may affect leukocyte typing results.

G. Bacterial, fungal or other contamination of any reagents may produce extraneous killing of lymphocytes in the test system or other nonspecific increase or decrease of reactivity.

H. CYNAP (Cytotoxicity Negative Absorption Positive) phenomena have been reported. This refers to antibody absorption by cells that are not killed in microlymphocytotoxicity testing.

I. The GenTrak Frozen Cell trays must be stored at -65^{0}C or colder throughout the dating period. NOTE: CONSTANT OPENING AND CLOSING OF A FREEZER MAY LEAD TO SUBSTANTIAL FLUCTUATIONS IN TEMPERATURE WHICH MAY IN TURN LEAD TO DECREASED VIABILITY OF THE FROZEN CELLS.

J. Exposure to carbon dioxide will lead to changes in pH of the media and subsequent problems with viability and/or reactivity. Once the package is opened, the GenTrak Frozen Cell Tray should never be placed on dry ice.

K. Reactivity of a given cell can only be judged by comparing the score of that cell's reactivity with the negative control serum and the test serum.

L. Since there is no current accepted U.S. standard for assignment of antibody specificity using a frozen cell tray, each laboratory should evaluate carefully its use of data derived from the GenTrak Frozen Cell Tray in conjunction with currently acceptable laboratory testing practices.

PERFORMANCE CHARACTERISTICS

A. All cells included on the GenTrak Frozen Cell Tray have been quality controlled to determine the HLA phenotype by available current methodologies.

B. The cells in each well should display a viability of 80-98%. If viability of less than 80% is noted, comments will be made on the front side of the worksheet.

C. All wells will display a reactivity score of 6 or 8 with the GenTrak positive control serum which is added to each well on the tray.

D. All wells will display a reactivity score of 1 or 2 with the GenTrak negative control serum which is added to each well on the tray.

E. The reagent complement controls in row 11 will display a reactivity score of 6 or 8 in the positive controls, and 1 or 2 in the negative controls.

F. Each production lot of cell trays demonstrates acceptable performance with a minimum of five known HLA antisera such that for each lot each cell has been tested for reactivity.

VIII. BIBLIOGRAPHY

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II. Procedure: D. Test. #4: Gravity settling the cells is the recommended procedure.
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EC REP

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