

SeraScreen

REF 0026-0803; FCT 30 - 10 Test (5 Trays of 2 Tests)

REF 0026-0804; FCT 60 - 5 Test (5 Trays of 1 Test)

**RUO** FOR RESEARCH USE ONLY**INTENDED USE**

For use in the detection of HLA-A, B or C antibodies in sera by a complement dependent micro-lymphocytotoxicity assay. This Device is not for detecting or determining tissue groups.

I. SUMMARY

The GenTrak Frozen Cell Trays contain 1 µL of isolated T-Lymphocytes derived from HLA phenotyped normal individuals; suspended in dimethyl sulfoxide (DMSO) diluted in RPMI media and Fetal Calf Serum. They may be used for primary screening of antisera HLA-A, B or C antibody detectable by the complement dependent microlymphocytotoxicity assay. Reagent component controls are provided in wells 12A-F on the tray.

II. PRINCIPLES

Isolated lymphocytes derived from normal HLA phenotyped individuals may be used for primary screening of antisera HLA-A, B or C antibodies, detectable by the complement dependent microlymphocytotoxicity assay.

Viable human lymphocytes possess HLA antigens on their surface, which may be recognized by anti-HLA antibodies present in the sera sample. With the addition of rabbit complement, cytotoxic changes may occur and be observed microscopically after addition of eosin or fluorescent dye. In a negative reaction, the lymphocytes are alive. In a positive reaction, the lymphocytes are dead.

III. REAGENT

- 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 10⁶/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.
- Standard Rabbit Complement is derived from a pool of normal rabbit serum.

IV. PRECAUTIONS

1. For Research Use Only, not for use in diagnostic procedures.
2. Do not use after expiration date.
3. A new test must be used for each sample. Do not use twice.
4. Components provided in the kit are approved for use in the Gen Trak's SeraScreen test. Do not use any other commercial kit component.
5. Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.
6. 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.
7. All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
8. The tests should be discarded in a proper biohazard container after testing.
9. The test must remain in the sealed pouch until used, use immediately after opening. Only open the pouch when the user is ready to perform the test.
10. Do not use a pierced or damaged pouch.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch at -70°C or colder. Do not refreeze. The test is stable until the expiration date marked on its sealed pouch. The test must remain in the sealed pouch until use. Thaw prior to use at room temperature (22°C ± 2°C) for 5-10 minutes. Do not subject complement to temperatures above 4°C. Use within one hour of thawing

VI. MATERIAL PROVIDED

- REF 0026-0803 FCT30: 5 trays of 2 tests (10 tests)
- REF 0026-0804 FCT60: 5 trays of 1 test (5 tests)
 - GenTrak Frozen Cell tray worksheets
 - Direction Insert.
 - GenTrak Rabbit Complement – frozen (2x2mL)

VII. MATERIAL REQUIRED BUT NOT PROVIDED

- Media for washing, e.g. RPMI-1640, MEM or McCoy's with 20% HIFCS

- Microliter syringes
- Pasteur pipettes
- Inverted phase contrast or fluorescent microscope
- Glass coverslides
- Fetal Bovine Serum
- Eosin Y or Fluorescent Dye
- Formalin
- Centrifuge

Optional Materials: SeraTrak Antibody Analysis Software system optional computer software supplied by GenTrak for the analysis of GenTrak Frozen Cell trays.

VIII. PROCEDURE**I. SPECIMEN COLLECTION AND PREPARATION**

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

- Draw blood according to accepted medical practice into a syringe, vacutainer or other suitable container.
- Blood is allowed to clot and then is centrifuged at 890 RCF for 10 minutes. If plasma is utilized, it must be converted to serum prior to testing.
- If testing is deferred, the serum should be stored at -70°C or colder until assayed.

I. TEST PROCEDURE

1. 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.** Remove the complement from the freezer and allow it to thaw. Do not subject complement to temperatures above 4°C.
2. Add 5 – 10µL of wash solution (RPMI-1640, MEM or McCoy's) to each well. Add the solution directly into the bottom of the well.
3. Allow cells to gravity settle for 10 minutes at 22°C ± 2°C.
4. Flick out the wash solution(RPMI-1640, MEM or McCoy's) 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!**
5. Add 2 µL of the serum test sample (antibody). **DO NOT ADD sera to reagent internal control wells 12A, B, C, D, E or F. Wells 12A and 12B contain internal positive control. 12C, 12D, 12E and 12F contain a pool of cells for internal negative control. (2 of these wells may be used for additional reagent testing.)**
6. Incubate for 30 minutes at 22°C ± 2°C.
7. Add 5 µL of Gentrak Rabbit Complement to the entire tray.
8. Incubate for 60 minutes at 22°C ± 2°C. Complement incubations may have to be adjusted according to the sensitivity desired.
9. Add 5 µL of fluorescent dye to the entire tray. Read tray after 15 minutes (for fluorescence).

Alternate Method:

- Add 3-5 µL aqueous eosin Y to entire tray. Incubate 3 minutes at room temperature (22°C ± 2°C).
- Add 5-8 µL of 37% neutralized Formaldehyde to the entire tray.
- Let tray settle 1 hour before reading.

IX. 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.**

X. 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.

Automated Analysis with Sera Trak:

Specific antibody 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 specific antibodies found in sera samples screened on frozen cell trays. The program allows:

1. The entry of panel members for defining any frozen cell tray designs.
2. The entry of all sera data, including demographics and final antibody assignments.
3. The entry of tray scores directly from the microscope into the computer system.
4. The entry of sera samples data, including demographics and user validated HLA types.

- The viewing of possible antibody specificities for a serum, based upon the automatic calculation of the tabulated tray scores entered for a serum.
- The printing of reports that indicated a 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.

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

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:

$$\text{R-VALUE} = \text{SQUARE ROOT} (\text{CHI-SQUARE}/\text{N})$$

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.

XI. QUALITY CONTROL

- Reagent complement internal controls, two each of positive (wells 12A and 12B) and negative (wells 12C and 12 D) reactivity are provided to assure the appropriate performance of the lymphocyte microcytotoxicity assay.
- Two additional wells are provided for reagent testing (wells 12E and 12 F).
- External positive controls could be run with each day's assay to demonstrate both adequate cell and complement reactivity.
- External negative controls could 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.
- For the use of external positive and negative control, add 2 µL through rows 1-10 only at step 6 of the Test Procedure.

XII. LIMITATIONS OF PROCEDURE

- 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.
- Improper thawing or washing may cause decreased viability by cell injury.
- HLA specificities are defined by compilation of data obtained through international exchange of antisera and leukocytes.
- Bacterial contamination or change in pH of the antisera may cause false reactions.
- CYNAP (Cytotoxicity Negative Absorption Positive) phenomena have been reported. This refers to antibody absorption by cells that are not killed in microlymphocytotoxicity testing.
- The GenTrak Frozen Cell trays must be stored at -65°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.
- 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.
- 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.
- 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.
- This test does not detect non cytotoxic antibodies.
- Insufficient specimen volumes may cause false negative reactions.

XII. PERFORMANCE CHARACTERISTICS

- All cells included on the GenTrak Frozen Cell Tray have been quality controlled to determine the HLA phenotype by available current methodologies.
- 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.
- All wells will display a reactivity score of 6 or 8 with the positive control serum which is added to each well on the tray.
- All wells will display a reactivity score of 1 or 2 with the negative control serum which is added to each well on the tray.
- The reagent complement controls in row 12 will display a reactivity score of 6 or 8 in the positive controls, and 1 or 2 in the negative controls.

XIV. BIBLIOGRAPHY

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-  For Research Use Only
-  Manufacturer
-  Batch code
-  Number of tests
-  Consult instructions for use
-  Use by
-  Catalog No.
-  Do not reuse
-  Upper limit of temperature
-  Biohazard

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