DATA SHEET

ADX MOLECULAR MEDICAL ADVISORY COMMITTEE
T&B CELL GENE REARRANGEMENT

METHODOLOGY

Molecular

TEST NAME

T&B Cell Gene Rearrangement
B Cell Gene Rearrangement
T Cell Gene Rearrangement

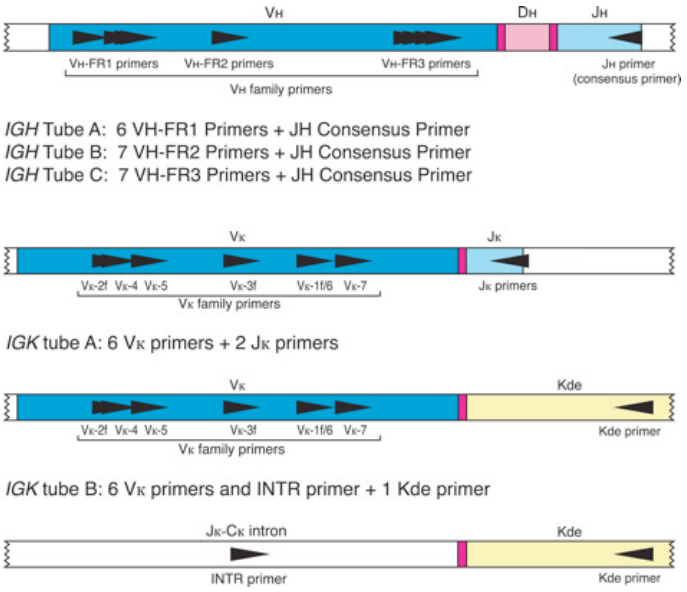
CLICK FOR REQUISITION

TEST DESCRIPTION

The T-cell receptor (TCR) genes (alpha, beta, delta, and gamma) are comprised of numerous, discontinuous coding segments that somatically rearrange to produce heterodimeric cell surface T-cell receptors, either alpha/beta (90%-95% of T cells) or gamma/delta (5%-10% of T cells). With rare exceptions (eg, some neoplastic B-lymphoid proliferations), other cell types retain the "germline" configuration of the TCR genes without rearrangement.

IGH + IGK B-Cell Clonality Assay

- For Identification of Clonal Immunoglobulin Heavy Chain and Kappa Light Chain Gene Rearrangements



BACKGROUND

Polymerase chain reaction (PCR) assays are routinely used for the identification of clonal B-cell populations. These tests amplify the DNA between primers that target the conserved framework (FR) and joining (J) regions (IGH Tubes A-C) of the immunoglobulin heavy chain (IGH), the variable (V) and joining (J) regions (IGK Tube A) of the immunoglobulin kappa light chain (IGK) and the variable, intragenic and Kappa Deleting Element (Kde) regions (IGK Tube B) of the immunoglobulin kappa light chain (IGK). These conserved regions lie on either side of an area within the V-J region where programmed genetic rearrangements occur during maturation of all B and T lymphocytes. The antigen receptor genes that undergo rearrangement are the immunoglobulin heavy chain & light chains genes in B-cells, and the T cell receptor genes in T-cells. Each B- and T-cell has a single productive V-J rearrangement that is unique in both length and sequence. Therefore, when this region is amplified using DNA primers that flank this region, a clonal population of cells yields one or two prominent amplified products (amplicons) within the expected size range. Two products are produced in cases when the initial rearrangement was non-productive and was followed by rearrangement of the other homologous chromosome. In contrast, DNA from a normal or polyclonal (many clones) population produces a bell-shaped curve of amplicon products (Gaussian distribution) that reflect the heterogeneous population of V-J region rearrangements.

Since the antigen receptor genes are polymorphic (consisting of a heterogeneous population of related DNA sequences), it is difficult to employ a single set of DNA primer sequences to target all of the con-

served flanking regions around the V-J rearrangement. N-region diversity and somatic mutation further scramble the DNA sequences in these regions. Therefore multiplex master mixes, which target several FR regions, are required to identify the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as prominent, single-sized products within the smear of different-sized amplicon products that form a Gaussian distribution around a statistically favored, average-sized rearrangement. As expected, primers that amplify from the different FR regions, which are located at three distinct regions along the heavy chain gene, produce a correspondingly different size-range of V-J products.

Gel electrophoresis is commonly used to resolve the different-sized amplicon products and ethidium bromide or other DNA intercalating dyes to stain and detect these products. A powerful alternative method is use of differential fluorescence detection with primers conjugated with fluorescent dyes that correspond to different targeted regions. Reaction products from several different master mixes can be pooled, fractionated using capillary electrophoresis, and detected simultaneously. This detection system results in unsurpassed sensitivity, single base resolution, differential product detection and relative quantification. In addition, the laboratory can eliminate the use of agarose and polyacrylamide gels, as well as the use of carcinogens such as ethidium bromide. Further, differential detection allows accurate, reproducible and objective interpretation of primer- specific products and automatic archiving of data. The limit of detection of this assay has been determined to be approximately 1 clonal cell in 100 hundred normal cells, and inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1-2 basepairs. This reproducibility and sensitivity allows monitoring and tracking of individual tumors during research or methods development. The automatic archiving of specimen data allows comparison of data collected at different times.

This test kit includes 6 master mixes. IGH Tubes A, B, and C target the framework 1, 2, and 3 regions (respectively) within the variable region, and the joining region of the IGH locus. IGK Tubes A and B target the variable, intragenic and joining regions of the IGK locus. The last master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

ASSAY USES

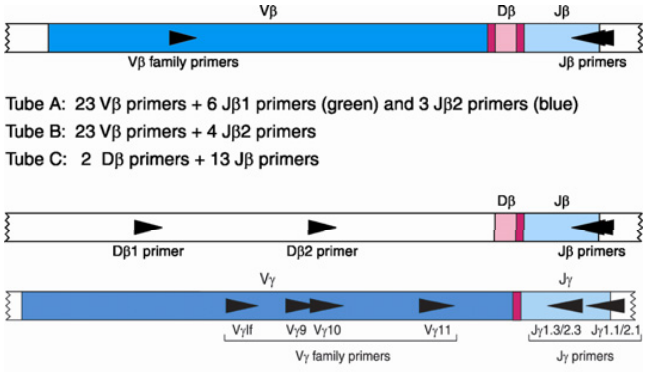
IMMUNOGLOBULIN HEAVY CHAIN AND KAPPA LIGHT CHAIN GENE REARRANGEMENT ASSAYS ARE USEFUL FOR THE STUDY OF:

- Identifying clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

TEST DESCRIPTION

TCRB + TCRG T-Cell Clonality Assay

- For Identification of Clonal T Cell Receptor Beta Chain and Gamma Chain Gene Rearrangements



BACKGROUND

TCRB Clonality Testing

The human TCR beta gene locus on chromosome 7 (7q35) includes 64-67 V genes belonging to 32 subgroups, 2 D segments, and 13 J segments, spread over 620 kilobases. The diversity of this locus has complicated PCR- based testing, and extended dependence on Southern blot analysis in many testing centers. However, this standardized multiplex PCR assay detects the vast majority of clonal TCR beta gene rearrangements using only 3 multiplex master mixes. The assay provides rapid TCR clonality assessment in 4-6 hours, reducing the number of Southern blot tests performed in the laboratory. The detection rate of clonal TCR beta gene rearrangements using this assay is unprecedentedly high. The performance characteristics of this assay have been independently determined by a European collaborative study involving 32 diagnostic PCR laboratories (BIOMED-2 Concerted Action) testing hundreds of clinical samples defined according to the WHO classification.

Three multiplex master mixes target conserved regions within the variable (V), diversity (D), and the joining (J) regions that flank the unique hypervariable antigen-binding region 3 (CDR3). TCRB Tube A contains 23 Vb primers + 6 Jb1 primers + 3 Jb2 primers. TCRB Tube B contains 23 Vb + 4 Jb2 primers. TCRB Tube C contains 2 Db + 13 Jb primers. PCR products can be analyzed by differential fluorescence detection using capillary electrophoresis or gene sequencing instruments, by heteroduplex analysis, or using standard gel electrophoresis with ethidium staining. Clonality is indicated if any one of the master mixes generates clonal band(s).

TCRG Clonality Testing

The human TCR gamma gene locus on chromosome 7 (7q14) includes 14 V genes belonging to 4 subgroups (6 are functional; 3 Open Reading Frames and 5 pseudogenes), 5 J segments, and 2 C genes spread over 200 kilobases. The diversity of this locus has complicated PCR-based testing, and extended dependence on Southern blot analysis in many testing centers. However, this standardized multiplex PCR assay detects the vast majority of clonal TCR gamma gene rearrangements using only 2 multiplex master mixes. The assay provides rapid TCR clonality assessment in 4-6 hours, reducing the number of Southern blot tests performed in the laboratory. The detection rate of clonal TCR gamma gene rearrangements using this assay is unprecedentedly high. The performance characteristics of this assay have been independently determined by a European collaborative study involving 32 diagnostic PCR laboratories (BIOMED-2 Concerted Action) testing hundreds of clinical samples defined according to the WHO classification.

Two multiplex master mixes target conserved regions within the variable (V) and the joining (J) regions that flank the unique hypervariable antigen-binding region 3 (CDR3). TCRG Tube A contains primers that target the V gamma 1-8 + V gamma 10 genes and all J gamma exon segments. TCRG Tube B contains primers that target the V gamma 9 + V gamma 11 genes and all J gamma exon segments. PCR products can be analyzed by differential fluorescence detection using capillary electrophoresis or gene sequencing instruments, by heteroduplex analysis, or using standard gel electrophoresis with ethidium staining. Clonality is indicated if any one of the master mixes generates clonal band(s).

This test kit includes 6 master mixes. TCRB Tubes A and B target framework regions within the variable region, and the joining region of the TCR beta chain locus. TCRB Tube C targets the diversity and joining regions. TCRG Tubes A and B target framework regions within the variable region, and the joining region of the TCR gamma chain locus. The last master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of the BIOMED tests. Many of our customers have remarked that this improves consistency and facilitates cross training on a broad range of different assays. These robust InVivoScribe assays can be used to test DNA extracted from virtually any source.

Polymerase chain reaction (PCR) assays are routinely used for the identification of clonal B- & T-cell populations. These tests amplify the DNA between primers that target the conserved framework (FR) and joining

(J) regions (Tubes A & B), or the diversity and joining regions (Tube C). These conserved regions lie on either side of an area within the V-J region where programmed genetic rearrangements occur during maturation of all B and T lymphocytes. The antigen receptor genes that undergo rearrangement are the immunoglobulin heavy chain & light chains genes in B-cells, and the T cell receptor genes in T-cells. Each B- and T-cell has a single productive V-J rearrangement that is unique in both length and sequence. Therefore, when this region is amplified using DNA primers that flank this region, a clonal population of cells yields one or two prominent amplified products (amplicons) within the expected size range. Two products are produced in cases when the initial rearrangement was non-productive and was followed by rearrangement of the other homologous chromosome. In contrast, DNA from a normal or polyclonal (many clones) population produces a bell-shaped curve of amplicon products (or Gaussian distribution) that reflect the heterogeneous population of V-J region rearrangements.

Since the antigen receptor genes are polymorphic (consisting of a heterogeneous population of related DNA sequences), it is difficult to employ a single set of DNA primer sequences to target all of the conserved flanking regions around the V-J rearrangement. N-region diversity and somatic mutation further scramble the DNA sequences in these regions. Therefore multiplex master mixes, which target several FR regions, are required to identify the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as prominent, single-sized products within the smear of different-sized amplicon products that form a Gaussian distribution around a statistically favored, average-sized rearrangement.
Gel electrophoresis is commonly used to resolve the different-sized amplicon products and ethidium bromide or other DNA intercalating dyes to stain and detect these products. A powerful alternative method is use of differential fluorescence detection with primers conjugated with fluorescent dyes that correspond to different targeted regions. Reaction products from several different master mixes can be pooled, fractionated using capillary electrophoresis, and detected simultaneously. This detection system results in unsurpassed sensitivity, resolution, differential product detection, and quantification. In addition, the laboratory can eliminate the use of agarose and polyacrylamide gels, as well as the use of carcinogens such as ethidium bromide. Further, differential detection allows accurate, reproducible and objective interpretation of primer-specific products and automatic archiving of data. The limit of detection of this assay has been determined to be approximately 5 clonal cells in 100 hundred normal cells, and inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1-2 basepairs. This reproducibility and sensitivity allows monitoring and tracking of individual tumors during research or methods development. The automatic archiving of specimen data allows comparison of data collected at different times.

This test kit includes 6 master mixes. TCRB Tubes A and B target framework regions within the variable region, and the joining region of the TCR beta chain locus. TCRB Tube C targets the diversity and joining regions. TCRG Tubes A and B target framework regions within the variable region, and the joining region of the TCR gamma chain locus. The last master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

ASSAY USES

ASSAY USES T CELL RECEPTOR BETA CHAIN AND GAMMA CHAIN GENE REARRANGEMENT ASSAYS ARE USEFUL FOR:

- Identifying clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas Monitoring and evaluation of disease recurrence Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

Results can be reported as “Positive” or “Negative” for “Detection of clonal T cell receptor beta chain or gamma chain gene rearrangement consistent with the presence of a clonal cell population”

SAMPLE INTERPRETATION

FOLLOWING THE ACCEPTANCE OF THE CONTROLS, THE CLINICAL SAMPLES ARE INTERPRETED AS FOLLOWS:

- One or two prominent peaks within the valid size range for TCRB Tubes A, B or C is reported as: "Detection of clonal T cell receptor beta chain gene rearrangement consistent with the presence of a clonal cell population."
- One or two prominent peaks within the valid size range for TCRG Tubes A or B is reported as: "Detection of clonal T cell receptor gamma chain gene rearrangement consistent with the presence of a clonal cell population."

SPECIMEN

ALL SAMPLES MUST BE TREATED AS POTENTIALLY INFECTIOUS MATERIAL. SPECIMEN MATERIAL IS HUMAN DNA EXTRACTED FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED SAMPLES.

WHEN CUTTING MULTIPLE BLOCKS, PLEASE USE DNA AWAY BETWEEN EACH BLOCK TO AVOID CROSS CONTAMINATION.

PLEASE USE POSITIVELY-CHARGED SLIDES AND 10% NBF FIXATIVE.

SEND FFPE BLOCK
OR
10-12 UNSTAINED SLIDES

SEND ALL SLIDES WITHIN 24-48 HOURS OF CUTTING FOR OPTIMAL DNA EXTRACTION.
PLEASE CUT FRESH UNSTAINED SLIDES WHEN TESTING IS REQUESTED.

SLIDE

- Pre-cut unstained slides from paraffin block in 5 micron sections.
- Air dry. Do not oven dry.
- Store specimen at room temperature (20-23.5°C).

STORAGE AND TRANSPORTATION

Use cold pack for transporting **blocks OR slides** making sure cold pack is not in direct contact with samples.

PLEASE SHIP TO:

Aurora Diagnostics
Attn: Molecular Department
5008 Mustang Rd.
Jacksonville, FL 32216

TURNAROUND TIME (TAT)

7 Days

CPT CODE

See chart Below

CPT CODE	ANTIBODY	DESCRIPTION
81261	IGH	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)
81264	IGK	IGK@, gene rearrangement analysis, evaluation to detect abnormal clonal population(s)
81340	TCRB	TRB@ (T cell antigen receptor, beta) gene rearrangement analysis to detect abnormal clonal population(s); using amplification methodology
81342	TCRG	TRG@ (T cell antigen receptor, gamma) gene rearrangement analysis to detect abnormal clonal population(s)

REFERENCES

References: IdentiClone TM TCRB + TCRG T-Cell Clonality Assay, Invivoscribe Technologies, Inc., San Diego, CA, 2015. Print
IdentiClone TM IGH + IGK B-Cell Clonality Assay, Invivoscribe Technologies, Inc., San Diego, CA, 2015. Print