T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Now Available for Gel Detection

Assay Uses

This Research Use Only assay identifies T-cell receptor gamma (TCRG) chain gene rearrangements and is useful for the identification of clonal T-cell populations and evaluation of new research and methods in malignancy studies.

Background

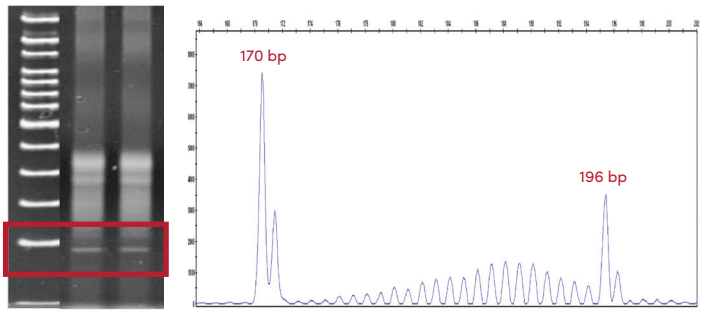
Invivoscribe's T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 represents an improved approach to PCR-based clonality testing of T-cell lymphoproliferative disorders. This multiplex PCR assay can detect the vast majority of TCR gamma gene rearrangements with a single multiplex master mix that includes primers for all known groups of TCR gamma variable region genes and joining region genes involved in rearrangements in T-cell lymphomas. Amplified products generated targeting the TCR gamma gene locus fall within a single size range to facilitate interpretation.

Specimen Requirements

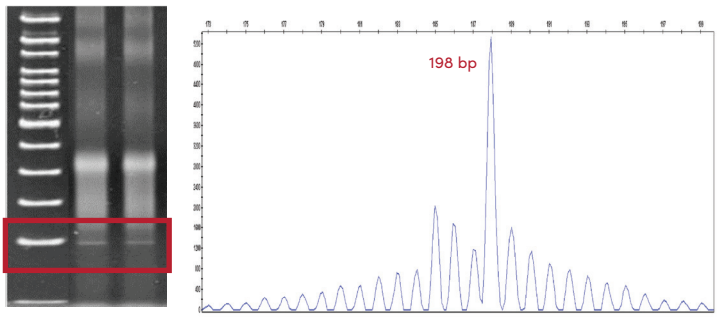
This assay tests extracted and purified genomic DNA (gDNA).

Cell lines containing clonal T-cell rearrangements and one polyclonal (negative) sample were assessed using the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 comparing detection methods. The data shown below was generated using 5% dilutions of DNA from each of the positive cell lines in polyclonal DNA or polyclonal DNA alone. Following PCR amplification, the products were detected on an ABI 3500xL instrument (right) versus heteroduplexing then running on a PAGE gel (left). The clonal products generated with the TCRG-6FAM master mix fall within a single contiguous size range of 159 bp to 207 bp (indicated by a red border):

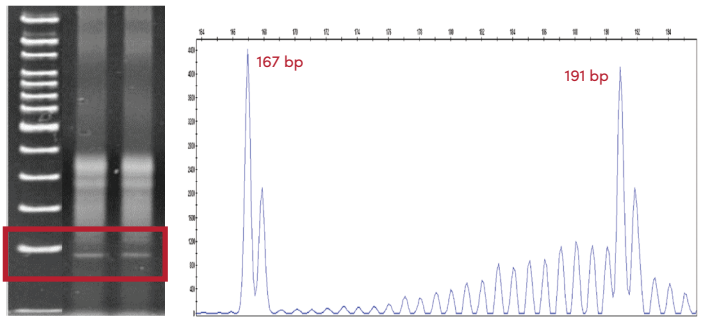
5% IVS-0005 Clonal Control DNA



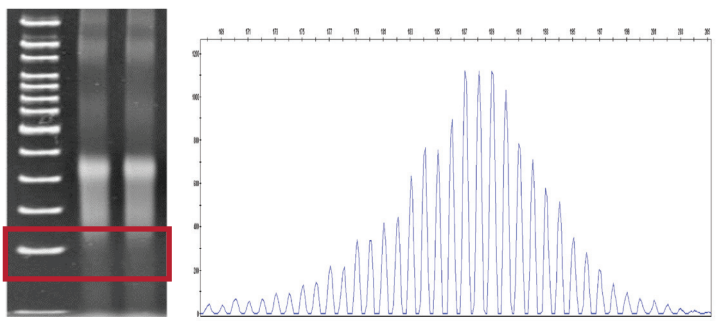
5% IVS-0009 Clonal Control DNA



5% IVS-016 Clonal Control DNA



IVS-0000 Clonal Control DNA



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Materials

The gel detection method requires additional reagents, not included in the kit:

Table 1. Materials Required (not provided)

Reagent/ Material	Recommended Reagents/ Materials & Suppliers	Part Number
Ethidium Bromide	Thermo Fisher Scientific: UltraPure™ 10mg/mL Ethidium Bromide	15585-001
6% Polyacrylamide Gels	Thermo Fisher Scientific: Novex® TBE Gels (6%, 12 well)	EC62652Box
TBE Running Buffer	Thermo Fisher Scientific: Novex® TBE Running Buffer (5X)	LC6675
Gel Loading Buffer	Thermo Fisher Scientific: 10X BlueJuice™ Gel Loading Buffer, Novex Hi- Density TBE Sample Buffer (5X)	10816-015, LC6678
100 bp DNA Ladder	Thermo Fisher Scientific: TrackIt™ 100 bp DNA Ladder	10488-058
Gel Electrophoresis Unit	N/A	N/A

Method

The gel detection protocol is consistent with the standardized method in all Invivoscribe gel detection assays:

Gel Detection – Heteroduplex Analysis

1. Denature 20 µL of PCR products at 94°C for 5 minutes.
2. Re-anneal PCR products at 4°C for 60 minutes.
3. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE) and 0.5X TBE running buffer.
4. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.
5. Load 20 µL of mixture into wells of the gel.
6. Run gel at 110V for 2-3 hours or 40-50V overnight.
   - Voltage and electrophoresis time depend on the PCR amplicon size, acrylamide gel thickness and type of PCR equipment.
   - Voltage and run time can be adapted accordingly.
7. Stain the gels in 0.5 µg/mL ethidium bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.
8. Destain the gels 2X in water for 5-10 minutes.
9. Visualize the gel using UV illumination.
10. Photograph the gel and interpret the data.

Ordering Information

Catalog #	Products	Quantity
1-207-0101	T-Cell Gamma Receptor Gene Rearrangement Assay 2.0	33 Reactions
1-207-0111	T-Cell Gamma Receptor Gene Rearrangement Assay 2.0 Mega Kit	330 Reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

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