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MyTREC® RealTime qPCR Assay Reagent Kit for Quantification of Human T-Cell Receptor Excision Circles (TRECs)

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MyTREC® RealTime qPCR Assay Reagent Kit

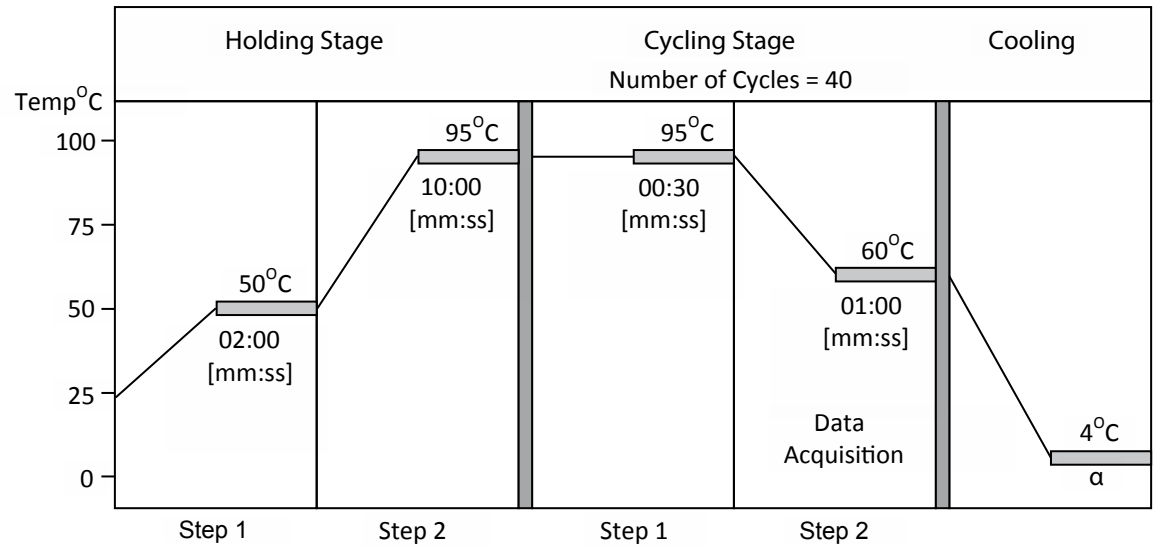
MyTREC® is a RealTime qPCR Assay Reagent Kit for quantification of Human T-Cell Receptor Excision Circles (TRECs).

Features of MyTREC® kit

1. All-In-One, Ready-To-Use RealTime qPCR Assay Reagent Kit
2. TaqMan® Probes for sensitive and accurate detection
3. Standard qPCR cycling conditions
4. Broad dynamic range of the calibrators for reliable testing of precious samples
5. qPCR Master Mix is available with (high/low ROX™) or without ROX™ to make it compatible for use with most of the RealTime PCR Instruments.

What is TREC?

TREC is a specific marker of T cells of thymic origin and thymic function can be determined by quantitative analysis of TRECs. TRECs are extra chromosomal DNA byproducts of T-Cell Receptor (TCR) rearrangement and "TCR delta deletion TREC" has been shown to be the most accurate TREC for measuring thymic



Graph 1 PCR program.

output. MyTREC® Assay specifically measures "TCR delta deletion TREC" using quantitative, RealTime PCR [1-3].

Applications of TREC Assay

Quantitative TREC analysis has a demonstrated clinical validity in:

6. Detection of Severe Combined Immunodeficiency (SCID) in newborns. SCID is the most severe and fatal variant of Primary Immunodeficiency diseases [4]. TREC Assay is also referred to as SCID Assay / test.
7. Detection of T cell lymphopenia [4].
8. Determination of thymic output/immune reconstitution following. Hematopoietic Stem Cell Transplantation (HSCT), antiretroviral therapy/cancer vaccines, thymic transplants [1, 5, 6].
9. Evaluating immune competence in patients with primary immunodeficiencies, idiopathic T cell deficiencies [7].

Description

GenenPlus introduces the MyTREC® RealTime qPCR Assay Reagent Kit for highly sensitive and accurate quantification of TRECs in a blood volume as little as 1 µl. The proprietary Calibrators have been optimized to provide exceptional efficiency and sensitivity with TaqMan® dual-labeled fluorescent probes. The TRECs and β-Actin (Reference Gene) are assayed in a singleplex format on any RealTime Instrument compatible with FAM™ and VIC® reporter dyes. The Kit is provided with PCR Master Mix, containing low/high/no ROX™ dye to enable RealTime PCR Instrument compatibility. GenenPlus is also developing RNAseP as an alternative reference gene calibrator, as some end users prefer RNAseP over β-Actin.

Table 1. Preparation of the reaction mix.

Component	One Rxn	100 Rxns
PCR Master Mix	10 µl	1000 µl
Primer/Probe	1 µl	100 µl
BSA	1 µl	100 µl
Nuclease-Free Water	4 µl	400 µl
Volume of Rxn Mix	16 µl	1600 µl

Specification

MyTREC® Assay detects 10 genome equivalent copies per reaction (Calibrator DNA dilution).

Specimen

Dried Blood Spot (DBS) or Whole Blood EDTA.

DNA is extracted from specimens prior to conducting the PCR.

Kit Contents

Calibrators (spanning a dynamic range of 6-$\log_{10}$ magnitude), Primers, TaqMan® Probes (FAM™/TREC, VIC®/β-Actin), PCR Master Mix **(Table 1)**, Positive Control, BSA, Nuclease-Free Water.

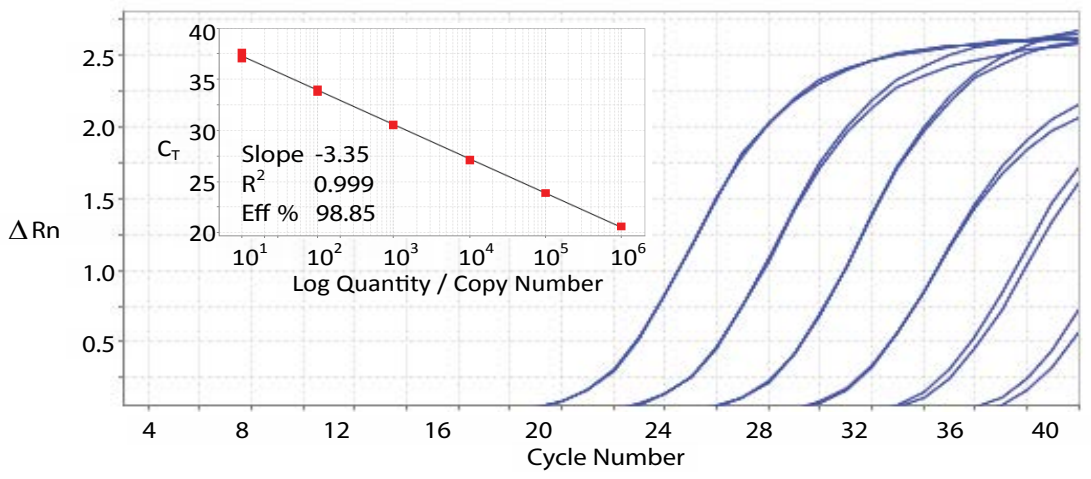
GenenPlus' MyTREC® Kit versus Perkin Elmer's Enlite™ Neonatal TREC Kit

MyTREC® Kit is a stand-alone Reagent Kit that can be used with any RealTime PCR Instrument compatible with FAM™ and VIC® dyes. Enlite™ TREC Kit requires the purchase of an Instrument (Fluorometer), WorkStation and Software along with the reagents to run the assay.

Mix gently, spin down briefly and transfer 16 µl to the well.

Add 4 µl of Calibrator DNA or Test Sample DNA, mix, seal and spin.

Always run Negative Control (Non-Template Control) and Positive Control (Positive Control DNA is provided with the Kit).



Graph 2 Typical results of MyTREC® Real-time qPCR Assay on StepOne™ Real-time PCR System.

Instrumentation Compatibility

MyTREC® Assay can be performed on all RealTime PCR Instruments that are compatible with FAM™, VIC® reporter dyes.

PCR Program

The Instrument is programmed as follows (Graph 1) before setting up the qPCR reactions and run according to Instrument operator's manual.

Reading and Analyzing the Results (Table 2, Graph 2 and Chart 1)

View results in the FAM™ channel (510 nm) for TREC and VIC® channel for β-Actin (β-Actin data is not shown here). Perform data analysis as described in the Instrument operator's manual.

MyTREC® real-time assay (Graph 2)

The Calibrators (range of 6-$\log_{10}$ magnitude) were amplified, in duplicate reactions with primers and TaqMan® FAM™ probe on StepOne™ RealTime PCR Instrument and data was analyzed using StepOne™ Software v2.3. Standard curve plot and linear regression statistics are shown in the inset along with the amplification plot.

Testing clinical samples using MyTREC® real-time assay (Chart 1)

De-identified newborn Dried Blood Spot specimens were processed for DNA extraction [4]. The samples were then tested in singlicate measurements on StepOne™ RealTime PCR Instrument and data was analyzed using StepOne™ Software v2.3. The SCID samples were successfully identified as SCID presumptive positive.

Disclaimer

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Table 2 Analyzing the data.

Test Sample	Positive Control	Non-Template Control	Result
No Amplification	C_T<37	Negative	TREC Negative
Amplification C_T<37	C_T<37	Negative	TREC Positive
No Amplification	Not Detectable	Negative	PCR Failure
Amplification Signal	Amplification Signal	Positive	PCR Contamination

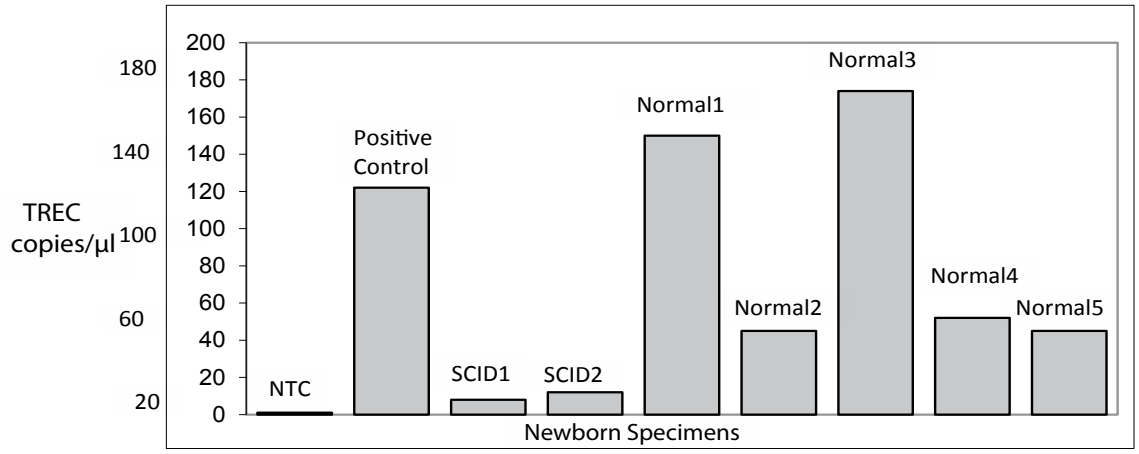

Chart 1 Clinical samples were assayed by MyTREC® RealTime qPCR Assay on StepOne™ RealTime PCR System.

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