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Products

MyTREC™ TREC Singleplex Real-Time qPCR Assay Kit (Human)

- GP-T10120100 ……. 100 x 20 µl Reactions
- GP-T10120500 ……. 500 x 20 µl Reactions

MyTREC™ Beta Actin Singlplex Real-Time qPCR Assay Kit (Human)

- GP-A20120100 ……. 100 x 20 µl Reactions
- GP-A20120500 ……. 500 x 20 µl Reactions

NEW: Rhesus macaque (*Macacus mulatta*)

TREC Singleplex Real-Time qPCR Assay Kit

- GP-MM50120100 ……. 100 x 20 µl Reactions
- GP-MM50120500 ……. 500 x 20 µl Reactions

Beta Actin Singleplex Real-Time qPCR Assay Kit

- GP-MM60120100 ……. 100 x 20 µl Reactions
- GP-MM60120500 ……. 500 x 20 µl Reactions

Upcoming Products

- MyTREC™ TREC / Beta Actin Duplex Real-Time qPCR Assay Kit

Rhesus macaque (*Macaca mulatta*) TREC/Beta Actin Singleplex Real-Time qPCR Assay Kits

For Research Use Only

Products' Specifications

- GP-MM50120100 / GP-MM60120100 100 x 20 µl Reactions
- GP-MM50120500 / GP-MM60120500 500 x 20 µl Reactions

Difference between GP-MM50120100 (or GP-MM60120100) and GP-MM50120500 (or GP-MM60120500)

GP-MM50120100 / GP-MM60120100 has components to run a total of 100 x 20 ul reactions that includes reactions for Calibrators (Standards), Non Template Control (NTC), Positive Control and Test Samples. Also, the Calibrators are in sufficient quantity for one Calibration curve, set up in triplicate reactions.

GP-MM50120500 / GP-MM60120500 has components to run a total of 500 x 20 ul reactions that includes reactions for Calibrators (Standards), Non Template Control (NTC), Positive Control and Test Samples. Also, the Calibrators are in sufficient quantity for five independent Calibration curves, set up in triplicate reactions.

Storage and Stability

All components of the kit should be stored at -20 to -30 deg C (constant temperature non-frost-free freezer). Avoid repeat freeze-thaws. Avoid exposure of components (probes, ROX®, Gene Expression Master Mix containing ROX®) to light. With proper storage and handling, the full activity of the kit is retained until the expiry date.

Description

The Rhesus macaque (*Macaca mulatta*) TREC Real -Time qPCR Assay Reagent Kit has been designed and developed for sensitive and accurate quantification of *Rhesus macaques's Signal Joint* TRECs (T-Cell Receptor Excision Circles) by **Absolute Quantification Method generating a Calibration / Standard Curve**. The proprietary Calibrators are "Ready-To-Use" and are serially diluted in a stabilizing diluent. The kit is formulated with TaqMan® dual-labeled fluorescent probes and a Gene Expression Master Mix containing antibody-mediated hot-start DNA Polymerase, Enhancers, Stabilizers, dNTPs, etc. A separate ROX® reference dye stock aliquot is provided for optional use and to enable instrument compatibility. **The kit is optimized for standard mode on fast qPCR instruments and standard cycling conditions on standard qPCR machines.**

Components	Volumes µl	
	GP-MM50120100 **GP-MM60120100**	**GP-MM50120500** **GP-MM60120500**
• Calibrators, **C**	15	75
• Gene Expression Master Mix, 2x, **MM**	1000	5000
• ROX® stock solution (light-sensitive), **ROX**	45	200
• Primers / Probe (light-sensitive), 20x, **P/P**	110	550
• Reaction Enhancer, 20x, **RE**	110	550
• Positive Control (TREC or Beta Actin), **PC**	15	75
• Nuclease-Free Water, **NFW**	1000	5 x 1000

Experiment Method

Real -Time qPCR Assay by Absolute Quantification using a Calibration / Standard curve

Calibrator copy numbers

- GP-MM50120100 / GP-MM50120500: TREC copies: 10^6, 10^5, 10^4, 10^3, 10^2, 25
- GP-MM60120100 / GP-MM60120500: Beta Actin copies: 10^7, 10^6, 10^5, 10^4, 10^3, 10^2

- Note: Have designated areas for Test Sample preparation, PCR set up and PCR run
- Note: Use a dedicated clean PCR area / PCR Workstation to set up the PCR.
- Note: Use sterile nuclease-free low adhesion barrier tips / other supplies for the PCR.
- Note: Before you thaw the Kit components, prepare your Test Samples and program the PCR Instrument for the run

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Protocol

1. Process your test samples for genomic DNA extraction and suspend the DNA in NFW. Freeze them until use.

2. Set up your Real-Time qPCR Instrument for the run, according to instrument's operator manual. The fields may vary with instrument.

- Set up a new experiment: Standard Curve (by Absolute Quantification), TaqMan® reagents, Standard Mode or Standard Cycling Conditions.
- Set up the qPCR plate by defining the targets and samples to the following Reporters / Quenchers

TREC = FAM™ (Reporter) / NFQ-MGB (Quencher) or
Beta Actin = VIC® (Reporter) / NFQ-MGB (Quencher)
Passive Reference Dye = ROX® (optional, depending on your instrument)

Note: Check if your PCR instrument supports FAM™ / VIC® reporters and/or requires calibration.

Note: If your PCR instrument does not give the option for choosing the quencher NFQ-MGB, check the operator's manual or call the manufacturer to know the alternative quencher parameters that could work equally well.

- Set up plate by assigning targets and samples to the wells in the plate as Standards, NTCs (Non-Template Controls), Unknowns. Assign the copy numbers for the Calibrator wells.

Note: Calibrator copy numbers

GP-MM50120100 / GP-MM50120500: TREC copies: 10^6, 10^5, 10^4, 10^3, 10^2, 25
GP-MM60120100 / GP-MM60120500: Beta Actin copies: 10^7, 10^6, 10^5, 10^4, 10^3, 10^2

- Set up qPCR cycling program on your PCR instrument as in Table 1:

Table 1: Cycling Protocol

Step	Cycles	Temperature	Time (hr:min:sec)
Polymerase Activation	1	95 deg C	00:03:00
Amplification	40		
Denaturation		95 deg C	00:00:30
Annealing/Extension		60 deg C	00:01:00
Hold	1	4 deg C	24:00:00

3. Thaw the Kit Reagents on ice (protect light sensitive components from light)

4. Give a gentle / brief vortex to thawed components and centrifuge briefly to collect the contents at the bottom of the tubes. During the reaction set up, if the components are sitting idle for a while, tap the tube / contents to mix and spin briefly.

5. Add ROX® reference dye to the Gene Expression Master Mix (Table 2). Check "Instrument Compatibility" on page 11 to know if your instrument is a high / low ROX® system. For instruments not listed, please check with your manufacturer.

Table 2: Amount of ROX® dye to add to the Gene Expression Master Mix

	ROX® Dye Volume (µl)	
	High ROX® dye system	Low ROX® dye system
To 1ml Gene Expression Master Mix (GP-MM50120100, GP-MM60120100)	40	4
To 5 ml Gene Expression Master Mix (GP-MM50120500, GP-MM60120500)	200	20

6. Make Reaction Mix (*Make Reaction Mix just before use. It cannot be stored*).

- Make enough Reaction Mix for the number of reactions needed plus 2-4 additional reactions to account for the pipetting errors.
- Set up Calibrator reactions in triplicate. Set up reactions for Test Samples, NTCs, Positive Control at least in duplicate.
- Set up a 1 x 20 µl Reaction Mix as in Table 3. Multiply the volumes by the "number of reactions" to scale up the Reaction Mix preparation.

Table 3: Reaction Mix set up.

Gene Expression Master Mix (2x)	10 µl
Primers / Probe (20x)	1 µl
Reaction Enhancer (20x)	1 µl
Nuclease-Free Water	4 µl
	16 µl

7. Dispense 16 µl aliquots of Reaction Mix into the wells of the qPCR plate that is compatible with your Real-Time PCR Instrument.

8. Add DNA template and make up the final reaction volume in the well with NFW (if needed) to 20 µl (see below). Pipette up and down twice gently to mix contents.

   - Calibrator wells : Add 4 µl Calibrator DNA
   - NTC wells : 4 µl NFW
   - Positive Control wells : 4 µl Positive Control DNA
   - Test Samples : up to 4 µl (we recommend using 100 pg – 500 ng DNA)

9. Seal the plate with optically transparent film.

10. Spin the plate briefly to settle the contents down and remove any air bubbles / or bring air bubbles to the top of the liquid.

11. Run the qPCR

    - Go to the experiment / cycling protocol (as in Table 1) you programmed previously on your PCR Instrument and start the run.

Note: Make sure the run is initiated before you walk away from the instrument.

12. Reading the results

    - The absorption range of fluorophores is seen in Table 4. View results in the channel specific for the FAM™ / VIC® dye.

Table 4: Absorption range of fluorophores

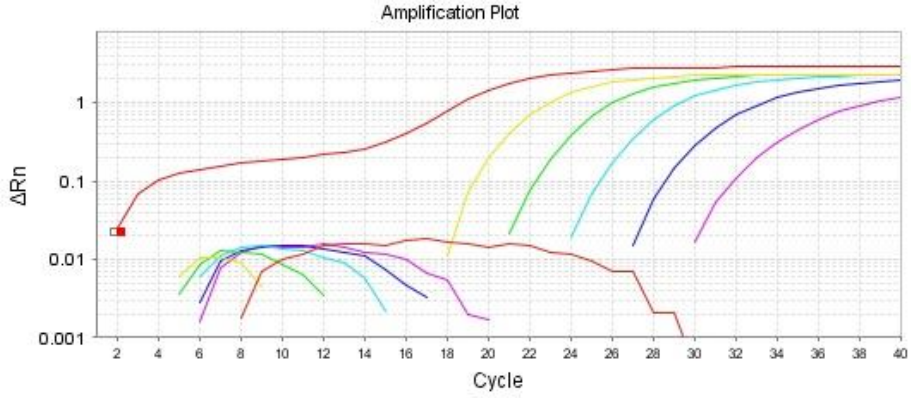
Dye	Absorption Max (nm)	Emission Max (nm)
6-FAM™	~ 495	~ 515
VIC®	~ 535	~ 555
ROX®	~ 573	~ 602

13. Analysis guidelines

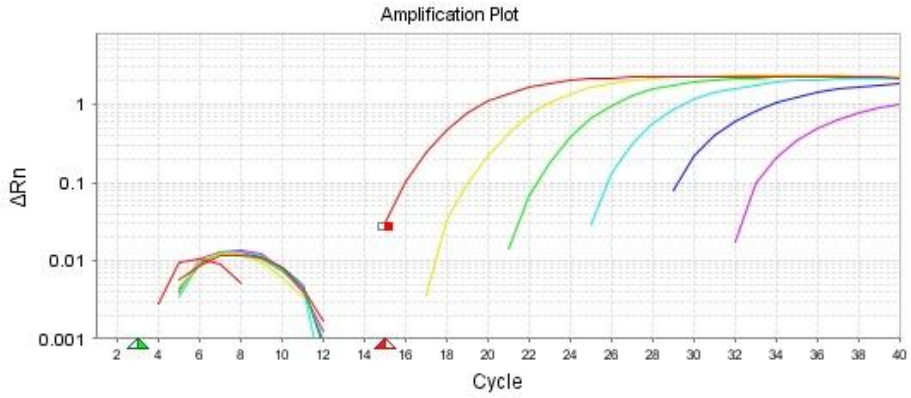
    - Perform data analysis as described in the instrument's operator's manual
    - **Some instrument software may need manual baseline and threshold settings for accurate analysis.**
    - Baseline: Based on TaqMan® probe chemistry, we recommend using **auto baseline** setting. However, verify if the baseline is set correctly: the end cycle should be set a few cycles before the cycle number where significant fluorescence signal is detected.
    - Automatic baseline sometimes can fail: For high copy number template (eg., 10^7 copies) the insrument's software fails to distinguish between noise and

true amplification resulting in auto baseline setting assigning an incorrect value for the baseline correction factor. In such cases, disable auto baseline or/and adjust the baseline manually.

Auto Baseline



Manual Baseline



- Threshold: Set Threshold correctly. Too low or too high threshold increases the standard deviation.
- **Auto Threshold** is set by the software. Verify if your auto threshold is set correctly.
- **Manual Threshold:** should be set in the geometric / exponential phase of the amplification curve, where the precision of replicates (Calibrators) is the highest.
- Evaluate the Standard Curve: Review the Slope, Amplification Efficiency (R^2), and individual Threshold Cycle (C_T) values.
- Slope / Amplification Efficiency Values: A slope close to -3.3 indicates optimal and 100% amplification efficiency. Make sure you run the Calibrators in triplicate to decrease pipetting inaccuracies.
- R^2 Value (Correlation Coefficient): An R^2 value > 0.99 is desirable. The R^2 value is a measure of the closeness of fit between the regression line and the individual C_T data points of the Calibrators.

- Amplification Plots: These plots help identify and examine irregular amplification, view threshold and baseline values for the run and locate any outliers.
- Correct the baseline and threshold values if necessary.
- Remove 1-3 (of the total 18 Calibrator measurements) outliers by omitting the wells.

14. Evaluating the Test Samples' Real-Time qPCR run

- Evaluate the amplification plots of all Test Samples. Any atypical plot should be rejected and retested.
- The Test Sample C_T and its analyte (TREC or Beta Actin) copy number is automatically obtained by the software from the standard curve plot.
- Analyze the Test Sample data as seen in Table 5.

Table 5: Data Analysis

Test Sample	Positive Control	NTC	Result
No Amplification	C_T<35	Negative	TREC / Beta Actin Negative
Amplification Signal	C_T<35	Negative	TREC / Beta Actin Positive
No Amplification	Not detectable	Negative	PCR Failure
Amplification Signal	Amplification Signal	Positive	PCR Contamination

15. Typical Results of Rhesus TREC Real-Time qPCR Kit on StepOne™ Real-Time PCR System

Figure 1A

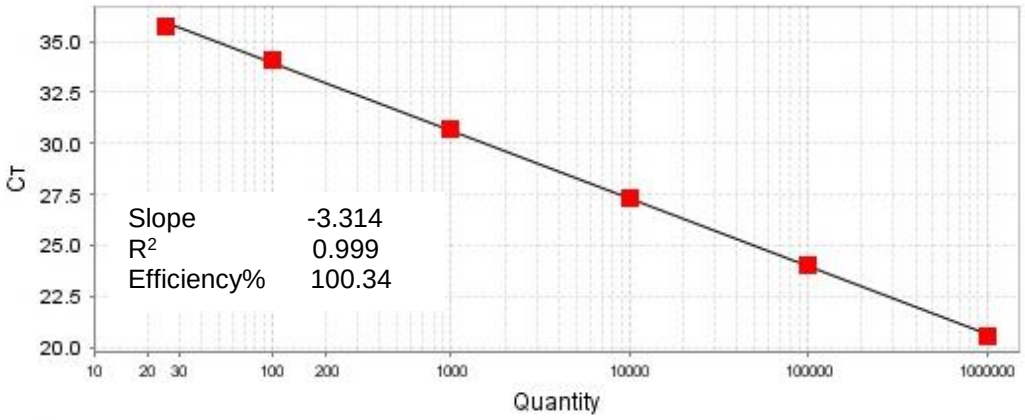


Figure 1B

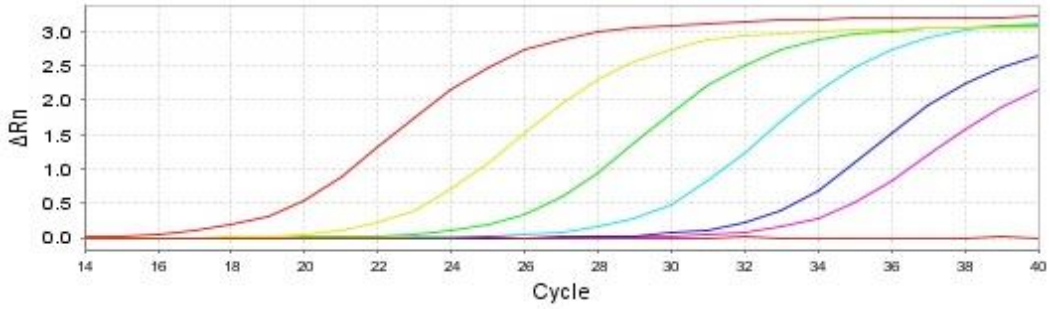


Figure1: The Calibrators (range of ~6-$\log_{10}$) were amplified, in singlicate reactions with primers, TaqMan® FAM™ probe and Gene Expression Master Mix on StepOne™ Real-Time PCR Instrument and data was analyzed using StepOne™ Software v2.3.
Standard Curve (Fig.1A) and linear regression statistics (Fig.1A) and Amplification Plot (Fig.1B) are shown.

16. Typical Results of Rhesus Beta Actin Real-Time qPCR Kit on StepOne™ Real-Time PCR System

Figure 2A

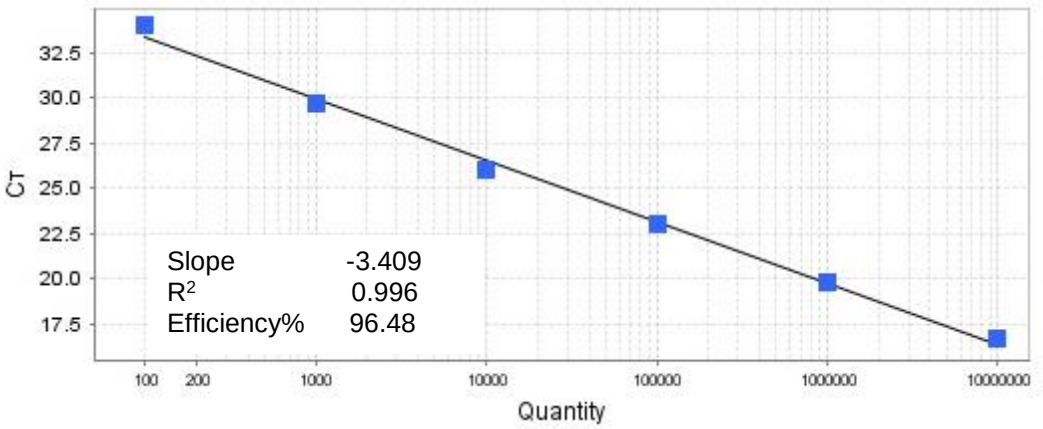


Figure 2B

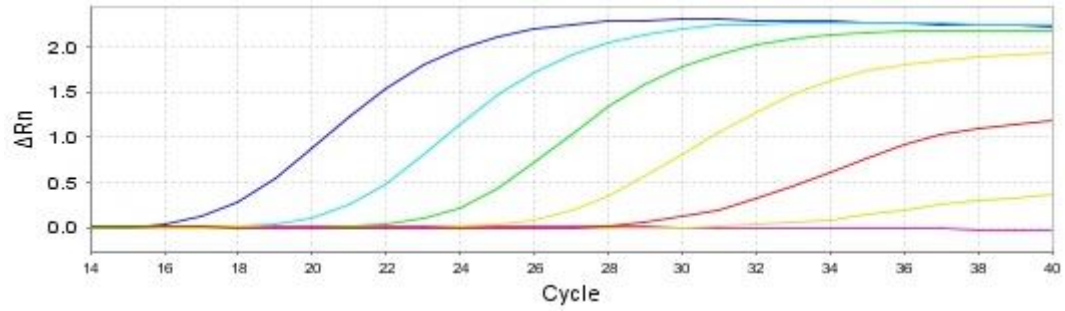


Figure2: The Calibrators (range of 6-$\log_{10}$) were amplified, in singlicate reactions with primers, TaqMan® VIC® probe and Gene Expression Master Mix on StepOne™ Real-Time PCR Instrument and data was analyzed using StepOne™ Software v2.3.
Standard Curve (Fig.2A) and linear regression statistics (Fig.2A) and Amplification Plot (Fig.2B) are shown.

17. TREC measurements and units

The final TREC measurements are reported in standard reporting units of either:

- TREC copies / 1 ml blood
- TREC copies / 1 million cells
- TREC copies / 1 µg DNA

Instrument Compatibility

Real-Time PCR Instruments

MyTREC™ Kits are compatible with various common Real-Time qPCR Instruments capable of recording FAM® and VIC® fluorescence. The following list is provided as a guideline for your reference. Call the manufacturer to ask about your instrument and know if your machine is compatible with a specific reporter dye and if it needs calibration for any particular dye. The list is not all inclusive.

High ROX

- Applied Biosystems 5700
- Applied Biosystems 7000
- Applied Biosystems 7300
- Applied Biosystems 7700
- Applied Biosystems 7900
- Applied Biosystems 7900HT
- Applied Biosystems 7900 HT Fast
- Applied Biosystems StepOne™
- Applied Biosystems StepOnePlus™

Low ROX

- Applied Biosystems 7500
- Applied Biosystems 7500 Fast
- Stratagene Mx3000P®
- Stratagene Mx3005P™
- Stratagene Mx4000™
- Applied Biosystems ViiA 7
- Applied Biosystems QuantStudio™
- Agilent AriaMx
- Douglas Scientific IntelliQube®
- QIAGEN Rotor-Gene Q

No ROX

- BioRad CFX
- BioRad iQ™
- BioRad Opticon™
- Roche LightCycler®