Sample Preparation for MyTREC™ Assay

Test Samples

The test samples: Dried Blood Spot (DBS) specimens, whole blood, Peripheral Blood Mononuclear Cells (PBMCs), cell suspensions, purified (flow-cytometry) cell populations, etc.

To determine the absolute quantity of TRECs in your test samples and to compare TREC measurements between test samples using the MyTREC Kit, the samples have to be processed first for genomic DNA. Process your test samples similarly for genomic DNA and suspend the DNA Extract in the same volume of Nuclease-Free Water / Buffer. Make sure the buffers do not contain PCR inhibitory elements.

Dried Blood Spot (DBS) Sampling and DNA Extraction

DBS Sampling and Extraction is a popular method to collect / process whole blood for TREC count analysis.

DBS Sampling

DBS sampling is popular and advantageous because of many factors: ease of obtaining sample by finger / heel stick, ease of transport and sample stability at ambient temperature or under refrigeration for many years.

About 10-20 ul of whole blood is spotted on a DBS card or approved filter paper cards (Whatman 903 filter paper). The spots are allowed to dry for 24 hours and then stored at 4 deg C (-20 deg C for long term storage) or processed.

DBS Sampling and Extraction is an alternative method to collect and process whole blood for TREC count analysis

<u>DNA Extraction from DBS / 96 well format</u>

Reference: Baker et al., Journal Allergy Clinical Immunology 2009 Vol 124 No 3 pg 522-527

1. A 3 mm punch is made on the specimen card and the punched disk is collected into 96 well Reaction Plate *
2. Make 5 empty punches on a paper towel to avoid cross contamination between samples
3. Add 90 ul Solution 1* to each well. Close the plate with Clear Adhesive Film *
4. Spin the plate at 3700 rpm 1' to immerse the disks
5. Leave the plate at RT for 15'
6. Spin at 3700 rpm 5'
7. Using a pipette, remove and discard (safely) the liquid
8. Repeat steps 3-7
9. Add 90 ul Solution 2 *
10. Spin at 3700 rpm 5'
11. Using a pipette, remove and discard the liquid

12. Add 24 ul Nuclease-Free Water
13. Place the plate at -20 deg C for 30' to overnight (overnight works better)
14. Take the plate out and let it warm to room temperature
15. Spin at 3700 rpm 1'
16. Place the plate in a thermal cycler for 25' 99 deg C
17. Remove the plate and let it cool down to room temperature
18. Spin 3700 rpm 1'
19. Store the DNA Extracts at -20 deg C until use
20. Use 4 ul (mix / pipette gently before taking an aliquot) of the DNA Extract to assay for the MyTREC™ Real-Time qPCR Assay

Note: please see the Product Manual for information on data analysis / reporting units.

***** *Supplies and Reagents for DNA Extraction from DBS*

- MicroAmp Optical 96-Well Reaction Plate (ABI)
- MicroAmp Clear Adhesive Film (ABI)
- Solution 1 = Generation DNA Purification Solution (QIAGEN)
- Solution 2 = Generation DNA Elution Solution (QIAGEN)

Alternative methods of genomic DNA prep for TREC Analysis

If you are not DBS sampling and using the above extraction method, we suggest the following kits of DNA extraction that are compatible with MyTREC™ Kits.

Suggested Kits for DNA extraction from Whole Blood, PBMCs, Cell Suspensions, Purified Lymphocytes:

- QIAamp® DNA Mini Kit (QIAGEN)
- GeneJET DNA Genomic DNA Purification Kit (ThermoFisher)
- ReliaPrep™ Blood gDNA MiniPrep System (Promega)
- Wizard Genomic DNA Purification Kit (Promega)