References:

The T-SPOT®.TB assay functions optimally with whole blood stored for up to 8 hours, but what happens if blood is stored for longer?

- Performing the T-SPOT.TB assay on blood stored for more than 8 hours may result in a decrease in spot counts and an increase in non-specific background in assay wells.

- When peripheral blood mononuclear cells (PBMCs) are separated using FICOLL-PAQUE®, the majority of the red blood cells and granulocytes are pelleted on the basis of density.

- When blood is stored for longer than 8 hours, granulocytes can become activated causing a decrease in their density. As a result, the granulocytes can contaminate the enriched PBMC layer.

- Granulocytes are known to become activated in some disease states (e.g. cancer).

- Activated granulocytes can release pre-stored granules, which may cause oxidative stress to lymphocytes in the PBMC layer. This may reduce cell viability and, more specifically, the ability of cells to release interferon-gamma.

- The presence of granulocytes may also result in non-specific background, affecting results with stored blood.

With stored blood granulocytes can appear in the PBMC layer.
The T-Cell Xtend reagent allows the T-SPOT.TB assay to be performed on blood samples stored for up to 32 hours without compromising accuracy.

The T-Cell Xtend reagent is an antibody complex which recognises CD66b, a specific cell surface marker of granulocytes, and cross-links the granulocytes to red blood cells.

This cross-linking increases the density of granulocytes so they pellet when applied to a density gradient.

The T-Cell Xtend reagent removes unwanted granulocytes from the sample.

Use of the T-Cell Xtend reagent has been demonstrated to reduce the presence of granulocytes in the PBMC layer, thus allowing prolonged storage of blood samples for up to 32 hours before performing the T-SPOT.TB assay.

Granulocyte contamination of PBMC layer increases with blood storage time.

Granulocyte contamination of PBMC layer is minimal with increased blood storage time and addition of the T-Cell Xtend reagent.
The T-Cell Xtend reagent is added to blood samples in the laboratory, immediately before commencing the T-SPOT.TB assay.

T-Cell Xtend may also be used with Leucosep tubes to simplify the FICOLL separation procedure.

Benefits of using the T-Cell Xtend reagent:

- Equivalent accuracy to standard T-SPOT.TB results but with added flexibility
- Samples can be collected at a distance from the testing laboratory
- Samples can be collected throughout the day or stored overnight
- Laboratory can perform T-SPOT.TB on blood samples received on 2 consecutive days at the same time
- Enables the use of cost-effective, overnight delivery service
- Removal of activated granulocytes may improve performance of the T-SPOT.TB assay in certain disease states (e.g. cancer)