TransFix®

Cellular antigen Stabilising Solution

Frequently Answered Questions

1. Where did TransFix® originate?

TransFix® was developed by scientists working for UK NEQAS. This organisation is the External Quality Assurance (EQA) assessor for all pathological diagnostic laboratories in the UK. UK NEQAS also supports many other laboratories in Europe and beyond.

Whole blood is very unstable due to proteolytic degradation. The result is that blood samples older than 36 hours are no longer viable for flow cytometric examination. For the same reason unstabilised whole blood is unsuitable for use as an EQA sample.

TransFix® was developed by UK NEQAS as a solution to this problem. When added to whole human blood it stabilises the profile and inhibits proteolytic deterioration.

TransFix®: A clinical sample stabilising fluid for use in clinical haematology and immunology.

D. Barnett, V.Granger, A.G. Pockley, J.M.saxton, I storie, L.Whitby & J.T.Reilly

Cytometry (1999) 38:88 (abstract)

Transportation of clinical samples between clinical sites raises concerns about sample integrity. TransFix® was developed as a stabilising solution with minimal dilution effect and which retains sample integrity for up to 10 days. This facilitates the flow cytometer and haematological analysis on normal, Leukaemia and HIV patients. Phase 1 studies examined 40 patients specimens and found no loss of antigenicity over a 10 day period for the following antigens: CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD19, CD20, CD22, Cd23, CD33, Cd34, CD45, CD79b, HLA-Dr and surface bound immunoglobulin. A full haematological profile (including differential) was obtainable at day 7.

2. How much TransFix® should I use per volume of blood?

For **HUMAN** samples the ratio of TransFix® to whole blood is 0.2ml per 1ml blood. Lower concentrations of TransFix® can be used but will reduce the maximum period of stability. Early publications mention a concentration of 0.1ml per ml of blood being used. This does not equate with the concentration of TransFix® now being sold.

TransFix® preserves the profile of the blood sample that exists at the time it is applied. If it is added to a sample that is 24 hours old the profile preserved will be for a 24 hour sample. Therefore, for the best results the blood sample should be freshly taken.

3. Has TransFix® been tested for other species?

Yes, it has been tested on mouse, rat, guinea pig, sheep, horse, pig and green turtle. The volume of TransFix® required to effectively stabilise 1ml of blood in different species varies considerably. In some species the volume required also varies by breed and type.

The user must determine the optimal dilution required by setting up a concentration curve, starting with 0.05ml per ml of whole blood and going up in 0.05ml steps to 0.4ml. Samples should be analysed before addition of TransFix® to establish a scatter diagram. The TransFix® treated samples should then be tested 10 days later. The optimal dilution is the concentration that gives the same scatter diagram as the original after 10 days storage at 4°C.

4. How should TransFix® be stored?

At room temperature (18 – 24ºC). TransFix® must not be stored at 4ºC and must **NEVER** be frozen.

5. How should TransFix® treated blood samples be stored?

Ideally, refrigerated at 4ºC. TransFix® treated samples are also stable at temperatures up to 22ºC for up to 7 days and at temperatures between 23ºC and 37ºC for 3 days. (Bergeron et al - Cytometry – 2002) Copies are available as a pdf download from the web site.

6. How do I tell if TransFix® has deteriorated?

TransFix® should not be used beyond the expiry date on the bottle. When TransFix® is contaminated it becomes cloudy. It is normally a clear pale green liquid.

7. Is TransFix® suitable for Immunophenotyping?

This is a complex question which is relies on several factors other than TransFix®, such as the type and manufacturer of antibodies being used. Cytomark recommend that potential users test the antibodies used in routine immunophenotyping to ensure there is no interference from TransFix®.

As a general guide TransFix® will protect the antigens listed below:

CD2, CD3, CD4, CD5, CD7, CD8, CD10, CS11B, CD13, CD14, CD19, CD20, CD22, CD23, CD33, CD34, CD45, CD79B and HLA-DR

8. Will TransFix® preserve intracellular antigens?

Yes, but it also causes a progressive permeabilisation of the cellular membrane. This means that the intracellular markers will leach out over time. As a general guide intracellular antigen measurement on TransFix® treated samples is only possible up to 3 days after the addition of TransFix®.

9. Does TransFix affect HIV Replication?

Evaluation of TransFix®, a commercial whole blood stabilizing reagent. This product reduces HIV replication.

Kim et al: Cytometry, Clinical Cytometry (2002) 50: 281

Samples treated with TransFix® retain morphology and cell surface expression over time. Shipping and handling of HIV+ samples involved inherent elevated costs and a risk of exposure to the HIV virus. The study showed that HIV+ blood samples showed a 1-log reduction in HIV replication as measured by HIV p24 antigen production. The results indicate that TransFix® has the ability to cause significant reduction in HIV replication.

10. Will TransFix® preserve cell lines

Yes, but the user must define the optimal concentration of TransFix® to use in their particular application.

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11. Can cells preserved with TransFix® be used for PCR?

Yes, but the user must define the optimal concentration of TransFix® to use in their particular application. See above.

12. Can cells preserved with TransFix® be used in Functional Assays?

No, TransFix is a preservative that fixates the cells. As a result they cannot be used in functional studies.

13. Does the type of anticoagulant used interfere with TransFix®?

No. TransFix® can be used in conjunction with all commonly used anticoagulants.

14. Does the lysis buffer used in flow cytometry matter?

Yes. Most commonly used red cell lysis buffers are compatible with TransFix®. Problems can occur if the lysis buffer is below room temperature when used. The buffer should be allowed to come to room temperature before application. Cal-Lyse works well in association with TransFix®

15. Can TransFix® be used to stabilise Bone Marrow?

Yes. A detailed protocol is available on the web site.

16. Can TransFix® be used to stabilise Fine Needle Aspirates?

Yes. A detailed protocol is available on the web site.

17. Can TransFix® be used to stabilise tissue biopsy samples?

There is no information available on this application.