

PRODUCT INFORMATION SHEET

Intended Use

TransFix/EDTA Cerebrospinal Fluid (CSF) Sample Storage Tubes are intended for collection and storage of human CSF specimens for characterisation of white blood cells by flow cytometry. Recovery of leucocyte subset markers can be accomplished over a 3-day period following stabilisation.

TransFix/EDTA CSF Sample Storage Tubes are Research Use Only products.

Summary and Principles

Subsets of leucocytes can be distinguished on the basis of cell surface antigens using fluorescent antibodies and flow cytometry.

Within CSF specimens, the qualitative and quantitative characterisation of infiltrated leucocytes via flow cytometry is an important tool for identification of various immunodeficiencies and hematologic diseases [1, 2]. However, the number of leucocytes present in CSF is often very low and degrade quickly. Therefore, assessment of these cells is reliant upon urgent analysis which is not always possible.

Addition of CSF to TransFix/EDTA CSF Sample Storage Tubes at or close to the time of lumbar puncture has been shown to significantly extend the integrity of these leucocytes, preserving the cell surface antigens until processing and analysis can be performed [1, 2].

TransFix/EDTA CSF Sample Storage Tubes consist of 5ml polypropylene vials containing 0.2ml TransFix/EDTA.

Reagents

TransFix is a clear green liquid containing paraformaldehyde and other cell preservatives. Refer to the SDS for safety and disposal information.

Precautions and Warnings

1. TransFix/EDTA CSF Sample Storage Tubes are Research Use Only products.
2. Incubation times or temperatures other than those specified may lead to erroneous results.
3. Do not use TransFix/EDTA CSF Sample Storage Tubes after the expiration date on the tubes and packaging.
4. Do not dilute or add other components to TransFix/EDTA CSF Sample Storage Tubes.
5. Do not use cell viability stains on samples treated with TransFix/EDTA CSF Sample Storage Tubes as they are fixed instantaneously.
6. Do not re-use TransFix/EDTA CSF Sample Storage Tubes.
7. TransFix/EDTA treated samples and all materials coming into contact with it should be handled as if capable of transmitting infection.
8. Avoid contact of TransFix/EDTA treated samples with the skin and mucous membranes. The cell preservative is considered an irritant and any contact should be washed off with soap and water immediately.
9. TransFix/EDTA does not contain any antimicrobial reagents. Microbial contamination should be avoided or erroneous results may occur.
10. SDS can be obtained at www.cytomark.com or by calling +44(0)1280 827460.

Indications of Product Deterioration

1. Cloudiness or precipitate visible in unused TransFix/EDTA CSF Sample Storage Tubes.
2. Colour change of TransFix from a clear green liquid in unused TransFix/EDTA CSF Sample Storage Tubes.
3. Reagent change from liquid to solid in unused TransFix/EDTA CSF Sample Storage Tubes.
4. If indications of product deterioration occur, do not use and contact Cytomark immediately at: cytomark@caltagmedsystems.co.uk

Storage Conditions and Stability

TransFix/EDTA CSF Sample Storage Tubes are supplied in sealed foil pouches.

Unused TransFix/EDTA CSF Sample Storage Tubes are stable at 2 - 8°C for up to 12 months or until the expiration date on the label.

Do not freeze TransFix/EDTA CSF Sample Storage Tubes.

Instructions for Use

1. Collect at least 1ml cerebrospinal fluid (CSF) by lumbar puncture according to the relevant Clinical Standard.

Note: CSF samples can be collected directly into the 5ml TransFix/EDTA CSF Sample Storage Tube, up to the 2.5ml graduation mark.
2. If necessary, carefully transfer 1 – 2ml CSF into the 5ml tube (up to the 2.5ml graduation mark) using a manual pipette as soon as possible.

Note: CSF samples must be transferred to the 5ml TransFix/EDTA CSF Sample Storage Tube within 2 hours of lumbar puncture.
3. Use a manual pipette to mix the TransFix-CSF solution and close the cap. Store / transport the 5ml TransFix/EDTA CSF Sample Storage Tube for up to 72 hours at 2 - 8°C.
4. After storage, open the cap and transfer all of the specimen into a flow cytometry tube (approx. dimensions 12 x 75mm) using a manual pipette.
5. Rinse out the 5ml TransFix/EDTA CSF Sample Storage Tube with 3ml PBS and transfer to the flow cytometry tube using a manual pipette.
6. Centrifuge the specimen at 540g for 5 minutes, at room temperature, with the brake off.
7. Aspirate the supernatant using a Pasteur pipette without disturbing the cell pellet and discard, leaving approx. 150µl cell suspension.
8. Carefully re-suspend the remaining cell suspension using a manual pipette. Stain the sample with the appropriate fluorochrome conjugated antibodies targeting cell surface antigens, according to the manufacturer's instructions.
9. Incubate for 15 minutes at room temperature, in the dark.

10. Add 3ml PBS to the specimen using a manual pipette, mix and centrifuge at 540g for 5 minutes, at room temperature, with the brake off.
11. Aspirate the supernatant using a Pasteur pipette without disturbing the cell pellet and discard, leaving approx. 150µl cell suspension.
12. Carefully re-suspend the remaining cell suspension with 150µl PBS using a manual pipette.

Note: Prior to CSF acquisition, run distilled water through the flow cell to remove any residual cells.
13. Acquire the specimen on a flow cytometer according to the manufacturer's instructions within 30 minutes of this preparation.

A certificate of analysis can be provided with every batch of TransFix/EDTA CSF Sample Storage Tubes.

Note: It is recommended that all antibody conjugates are validated in association with TransFix/EDTA CSF Sample Storage Tubes prior to use. Samples are available on request and a list of antibodies validated by Cytomark can be found on www.cytomark.com.

Note:

- a. Light scatter positions of cells stabilised by TransFix/EDTA may differ slightly from those of untreated cells.
- b. The dilution factor must be accounted for when calculating absolute cell counts. This can be done by multiplying the value given by the manufacturer to the absolute counting beads accordingly so that absolute cell counts are automatically corrected for TransFix/EDTA treated samples.

References

1. Use of TransFix Cerebrospinal Fluid Storage Tubes Prevents Cellular Loss and Enhances Flow Cytometric Detection of Malignant Haematological Cells after 18 Hours of Storage. De Jongste et al, Clinical Cytometry, 2013, 000B:00–00.
2. Guidelines on the use of Multicolour Flow Cytometry in the Diagnosis of Haematological Neoplasms. Johansson et al, British Journal of Haematology, 2014, 165, 455-488.

Ordering Information

Please call Cytomark at +44(0)1280 827460 for assistance. Additional information can be found online at www.cytomark.com.

Glossary of Harmonised Symbols

REF	Catalogue Number	Use by	LOT	Batch Code
Manufacturer		Temperature Limitation	RUO	Research Use Only
Consult Instructions For Use		Do not re-use	Biological Risk	
Irritant		Suspected Carcinogen		

Cytomark, a Division of Caltag Medsystems Ltd.
 Caltag Medsystems Ltd, Buckingham, MK18 1TF, UK