

PRODUCT INFORMATION SHEET

Intended Use

TransFix®/EDTA Cerebrospinal Fluid (CSF) Sample Storage Tubes are intended for stabilisation 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

Within CSF specimens, the qualitative and quantitative characterisation of infiltrated leucocytes via flow cytometry is an important tool for identification of various immunodeficiencies and haematologic 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.

Subsets of leucocytes can be distinguished by cell surface antigens using fluorescent antibodies and flow cytometry. The TransFix stabiliser acts by preserving cell surface antigens of lymphocyte subsets until processing and analysis can be performed.

Addition of CSF to TransFix/EDTA CSF Sample Storage Tubes at 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 formaldehyde and other chemicals.

Disposal

TransFix (EU Waste Code 18-01-06) contains formaldehyde which should be disposed of in accordance with local regulations. Avoid disposing into drainage systems and the environment.

Once the TransFix 'sample storage tube' contains a biological sample it must be considered an 'Absolute Hazard' (EU Waste Code 18-01-03) and disposal is in accordance with local regulations regarding clinical waste.

Precautions and Warnings

1. TransFix/EDTA CSF Sample Storage Tube is intended for use as specified in this document. It is a 'Research Use Only' product for professional use only.
2. Treat the sample with TransFix as quickly as possible to ensure the best results.
3. Do not use after the expiration date on the tubes and packaging.
4. Do not dilute or add other components to the tube before use.
5. Do not use cell viability stains on samples treated with TransFix as they are fixed instantaneously.
6. Do not re-use.
7. TransFix treated samples and all materials coming into contact with it should be handled as if capable of transmitting infection.
8. Avoid contact of TransFix treated samples with the skin and mucous membranes. The cell preservative is an irritant and any contact should be washed off with soap and water immediately.
9. TransFix does not contain any antimicrobial reagents. Microbial contamination should be avoided, or erroneous results may occur.
10. SDS can be obtained at www.cytomark.co.uk or by calling +44(0)1280 827460.

Indications of Product Deterioration

1. Cloudiness or precipitate visible in the TransFix.
2. Colour change of TransFix from a clear green liquid.
3. TransFix change from liquid to solid.
4. If indications of product deterioration occur, do not use and contact Cytomark immediately on: +44(0)1280 827460 or support@cytomark.co.uk.

Storage Conditions and Stability

TransFix CSF products are validated for shipping at -5 to 25°C for up to 7 days. Packaging insulation or cold packs are required for shipment outside of these conditions. TransFix products are supplied in sealed foil pouches.

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.

Instructions for Use

1. Collect at least 1ml cerebrospinal fluid (CSF) by lumbar puncture according to the relevant clinical standard.
2. Carefully transfer 1 – 2ml CSF into the 5ml tube using a manual pipette as soon as possible and no later than 2 hours after lumbar puncture.
3. Gently mix the TransFix treated sample and close the cap.
4. Store / transport the TransFix treated sample for up to 72 hours at 2 - 8°C.
5. After storage, open the cap and transfer all the specimen into a flow cytometry tube (approx. dimensions 12 x 75mm) using a manual pipette.
6. Rinse out the 5ml TransFix/EDTA CSF Sample Storage Tube with 3ml PBS and transfer to the flow cytometry tube using a manual pipette.
7. Centrifuge the specimen at 540g for 5 minutes, at room temperature, with the brake off.
8. Aspirate the supernatant using a Pasteur pipette without disturbing the cell pellet and discard, leaving approx. 150µl cell suspension.
9. 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.
10. Incubate for 15 minutes at room temperature, in the dark.
11. 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.
12. Aspirate the supernatant using a Pasteur pipette without disturbing the cell pellet and discard, leaving approx. 150µl cell suspension.
13. Carefully re-suspend the remaining cell suspension with 150µl PBS using a manual pipette. Prior to CSF acquisition, run distilled water through the flow cell to remove any residual cells.
14. Acquire the specimen on a flow cytometer according to the manufacturer's instructions within 30 minutes of this preparation.
15. **When treated with Transfix the dilution factor must be accounted for when calculating absolute cell counts. For a sample size of 1ml adjust the absolute cell count by multiplying the output by 1.2. For a sample size of 2ml adjust the absolute cell count by multiplying the output by 1.1.**

Notes:

1. Use caution when implementing automatic gating strategies as light scatter positions of cells stabilised by TransFix may differ from those of untreated cells.
2. 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.
3. A certificate of analysis can be provided with every batch of TransFix/EDTA CSF Sample Storage Tubes.

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 on +44(0)1280 827460 or email support@cytomark.co.uk for assistance. Additional information can be found online at www.cytomark.co.uk.

Product Description	Catalogue Numbers
TransFix / EDTA CSF Sample Storage Tube (2x 5ml tube)	TF-CSF-5-2
TransFix / EDTA CSF Sample Storage Tube (10x 5ml tube)	TF-CSF-5-10
TransFix / EDTA CSF Sample Storage Tube (25x 5ml tube)	TF-CSF-5-25
TransFix / EDTA CSF Sample Storage Tube (50x 5ml tube)	TF-CSF-5-50

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