

Technical Data Sheet

ICC Fixation Buffer

Product Information

Material Number: 550010
Size: 100 mL

Description

ICC Fixation Buffer is useful for fixing unstained cells that have been adhered to microscopic slides for cytokine immunocytochemical staining. This fixation buffer is intended to preserve human and rodent lymphoid cells for the subsequent immunocytochemical staining of intracellular cytokines. Once fixed the slides may be stored at -80°C for up to two months. When stored under those conditions no changes in cell morphology have been observed and the levels of intracellular cytokine detected by immunocytochemistry remain unaffected.

Note: The suitability of fixation conditions for cells undergoing immunocytochemistry staining depends on the availability of compatible antibodies that can specifically detect their cognate antigens under the same fixation conditions. With respect to intracellular cytokines and chemokines, BD Pharmingen offers a panel of purified anti-cytokine antibodies optimized for use in immunocytochemistry of cells fixed with formalin.

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

Immunocytochemistry (cytospins)	Routinely Tested
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Recommended Assay Procedure:

Procedure for using the Cytokine ICC Fixation buffer in Immunocytochemistry:

1. Harvest cells and wash them twice in PBS using centrifugation (400 x g for 5 min) to remove residual protein.
2. Adjust the cell concentration at $4-5 \times 10^6$ cells/ml in PBS.
3. Place 20 μ l of the cell suspension in each well of the adhesion slides and let them adhere at room temperature (RT) for 20 min. Please note that the slides should be washed in PBS at RT for 5 min before transferring the cells.
4. Fix cells on slides using ICC Fixation Buffer (Cat No. 550010) for 15 min at RT.
5. Wash slides 2X in PBS with 5 min incubations.
6. Block slides with PBS supplemented with 1% (w/v) BSA (Sigma) for 30 min at RT or 10 min at 37°C.
7. Wash slides 2X in PBS and proceed with staining or air dry them and store them at -80°C for future use.
8. Incubate slides with 20 μ l of 1% goat serum and PBS with 0.1% (w/v) saponin for 30 min at RT.
9. Wash slides 2X with PBS with 5 min incubations.
10. Block endogenous peroxidase activity with Endogenous Peroxidase Blocking Buffer (20 μ l/well) for 10 min at RT.
11. Wash 2X in PBS with 5 min incubations.
12. Incubate each well with Avidin (20 μ l/well) for 15 min.
13. Wash 2X in PBS with 5 min incubations.
14. Incubate each well with Biotin (20 μ l/well) for 15 min.
15. Wash 2X in PBS with 5 min incubations.
16. Incubate each well for 1 hr at RT with 20 μ l of purified cytokine-specific antibody or appropriate immunoglobulin isotype control diluted in Antibody Diluent Buffer for IHC (Cat. No. 559148).
17. Wash slides 2X in PBS with 5 min incubations.
18. Incubate each well with 20 μ l of a biotinylated secondary antibody diluted in Diluent Buffer (Cat. No. 559148) for 30 min at RT.
19. Wash 2X in PBS with 5 min incubations.
20. Apply 20 μ l of Streptavidin-HRP (Cat. No. 550946) to each well on slides and incubate for 30 min at RT.
21. Wash slides 2X with PBS with 5 minutes incubations.
22. Incubate with 3-3'-Diaminobenzidine tetra hydrochloride (DAB), (Cat. No. 550880) for less than 5 min at RT.
23. Stop the development of the color reaction by washing with PBS.
24. The slides are subsequently mounted in short-term storage mounting medium.

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Cytospins

1. Assemble the Cytospin's sample chamber (e.g. Cytospin 3, Shandon, UK or comparable centrifuge), filter card, slide and cytospin racks according to manufacturer's specifications.
2. Load 40 µl of approximately 1×10^6 cells to each sample chamber.
3. Spin slides at 600 rpm for 2 min.
4. Take slides out of the cytospin rack and place them on a staining rack.
5. For fixation and staining please follow the steps 4 through 24 specified above for staining cells on adhesion slides.

Danger: ICC Fixation Buffer contains 2.09% formaldehyde.

Hazard statements

May cause an allergic skin reaction.

Suspected of causing genetic defects.

May cause cancer. Route of exposure: Inhalative.

Precautionary statements

Wear protective clothing / eye protection.

Wear protective gloves.

Avoid breathing mist/vapours/spray.

If skin irritation or rash occurs: Get medical advice/attention.

IF ON SKIN: Wash with plenty of water.

Dispose of contents/container in accordance with local/regional/national/international regulations.

Suggested Companion Products

Catalog Number	Name	Size	Clone
559148	Antibody Diluent for IHC	125 mL	(none)
550946	Streptavidin HRP	50 mL	(none)
550880	DAB Substrate Kit	500 Tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

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