Technical Data Sheet

Fixation Buffer

Material Number: 554655
Size: 100 mL

Description

BD Cytofix™ Fixation Buffer is intended to preserve human and rodent lymphoid cells for the subsequent immunofluorescent staining of intracellular cytokines. BD Cytofix can also be used to preserve the light-scattering characteristics and fluorescence intensities of human and rodent hematopoietic cells that have been stained by immunofluorescence for subsequent flow cytometric analysis.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Recommended Assay Procedure:

1. Pellet 10^6 suspended cells (e.g., cytokine-producing cells generated by stimulatory culture) by centrifugation (250 - 300 x g) and carefully remove supernatants to avoid cell loss.
2. Add either 200 µl (for microwell plates) or 500 µl (for tubes) aliquots of cold DPBS containing protein and NaN3, gently resuspend cells, pellet, and remove supernatants.
3. Repeat step 2.
4. Add either 100 µl (for microwell plates) or 250 µl (for tubes) aliquots of fixation buffer to each cell pellet and resuspend the cells by either pipetting or vortexing. Incubate the cells with fixation buffer for 15 to 30 min at 4°C. (Cell aggregation can be avoided by vortexing prior to the addition of the fixation buffer.)
5. Fixed cells should be washed and suspended in a buffer that contains protein and NaN3, e.g., either Stain Buffer (FCS) [Cat. No. 554656] or Stain Buffer (BSA) [Cat. No. 554657]. Store the fixed cells at 4°C (protected from light) for subsequent immunofluorescent staining of intracellular cytokines. It is recommended that fixed cell samples be read as soon as possible, i.e., within one week.

For the immunofluorescent staining of intracellular cytokines, cells that have been previously fixed with BD Cytofix™ can be washed two times in a buffer that contains protein and NaN3 followed by incubating the cells for at least 10 minutes (4°C) in a buffer containing the cell-permeabilizing agent, saponin. BD Perm/Wash™ buffer (Cat. No. 554723) is ideally suited for this purpose. The fixed and permeabilized cells can then be stained for intracellular cytokines.

Procedure for fixing immunofluorescently-stained cells with BD Cytofix™:

Cells stained by immunofluorescence for cell surface antigens can be fixed as described above and stored (4°C, protected from light) for subsequent analysis by flow cytometry (or fluorescence microscopy).

Note: BD Cytofix/Cytoperm™ solution (Cat. No. 554722) and the BD Perm/Wash™ buffer (Cat. No. 554723) are included in BD Cytofix/Cytoperm Kit (Cat. No. 554714) as well as the BD Cytofix/Cytoperm Plus Kit with GolgiStop™ (containing monensin; Cat. No. 554715) and BD Cytofix/Cytoperm Plus Kit with GolgiPlug™ (containing brefeldin A; Cat. No. 555028).
Danger: BD Cytofix™ Fixation Buffer contains 4.21% formaldehyde (w/w).

Hazard statements

Harmful if inhaled.
Causes skin irritation.
Causes serious eye damage.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalative.

Precautionary statements

Obtain special instructions before use. Do not handle until all safety precautions have been read and understood.
Wear protective clothing / eye protection/ gloves.
Contaminated work clothing should not be allowed out of the workplace. Wash contaminated clothing before reuse.
Do not breathe mist/vapours/spray.
If exposed or concerned: Get medical advice/attention.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If skin irritation or rash occurs: Get medical advice/attention.

Storage Statement
Store locked up.

Disposal Statement
Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Suggested Companion Products

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
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<td>Stain Buffer (FBS)</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
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<tr>
<td>554723</td>
<td>Perm/Wash Buffer</td>
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<td>BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit</td>
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<tr>
<td>555028</td>
<td>BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)</td>
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Product Notices

Please refer to wwwbdbiosciences.com/pharmingen/protocols for technical protocols.

References