

BD FACSelect™ Buffer Compatibility Resource

Features

Quickly find experimental results for monoclonal antibodies tested under various fixation and permeabilization conditions

Data are available for intracellular and surface marker specificities

Both human and murine primary cell data are available

Real results to help optimize multicolor panels

Intracellular phosphoprotein detection using flow cytometry requires that cells be treated with fixation and permeabilization buffers that may alter native proteins. However, different intracellular and cell surface proteins frequently require different conditions for optimal detection. This creates a challenge for designing multicolor panels for analyzing multiple protein types in a single sample. The BD FACSelect™ buffer compatibility resource, powered by Cytobank, allows users to find staining results for many relevant intracellular and surface proteins.

Selection of appropriate fixation and permeabilization buffers is critical for simultaneous analyses of phosphoproteins, transcription factors, surface markers, and other proteins involved in apoptosis or cell cycle. BD Biosciences offers a broad portfolio of fixation and permeabilization buffers to meet the diverse needs of our research customers. The BD FACSelect buffer compatibility resource provides real experimental results for cells that have been processed using different variables including blood source, fixation buffer, permeabilization buffer, fluorochrome, staining sequence, and antibody titration. Use these results to jump start assay design, saving time and avoiding the expense of purchasing reagents for trial-and-error optimization experiments.

Figure 1. Antibody landing page.

Quickly find antibody specificities by searching keywords. Customize search results by selecting or clearing lasers or fluorochromes. Sort results by target, clone, species, antigen localization (surface vs intracellular), cell source, or staining protocol. Click links to take you to product information, state-of-the-art sample preparation protocols, or to specific antibody staining results.

Readily accessible results and protocols from a landing page

Users can quickly navigate from the antibody landing page to specific reagent data sheets and protocols with a single mouse click. Each data sheet displays experimental results by fluorochrome and permeabilization buffer used. For intracellular phosphoprotein analysis, results are shown as histogram overlays for stimulated and non-stimulated sample conditions. By browsing real experimental results, researchers can easily determine the best fluorochrome and buffer combinations to use for their human and mouse cell experiments. Find all state-of-the-art cell preparation protocols on the antibody landing page under the protocols tab. Follow the link to additional protocols to find suggested stimulation conditions.

The best staining strategy and antibody titration made simpler

Surface marker staining can be compromised by the fixation and permeabilization process. To facilitate the optimization of post-permeabilization surface marker staining, data are provided for multiple fluorochrome conjugates, clones, and concentrations of each antibody specificity. For surface markers that cannot be resolved using post-permeabilization staining methods, two alternative staining protocols are available in addition to the corresponding results.

Visit bdbiosciences.com/phosflow for more information.



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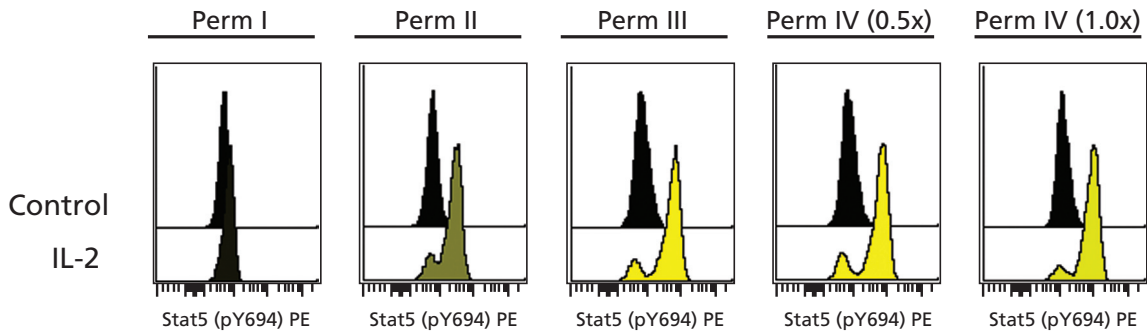


Figure 2. Perm buffer choice impacts the ability to resolve activated (phosphorylated) proteins from basal levels of activation.

To induce protein phosphorylation, human PBMCs were stimulated at 37°C with 100 ng/mL of recombinant human IL-2 for 15 minutes. Cells were fixed using BD Cytotix™ fixation buffer and permeabilized using BD Phosflow™ perm buffer I, II, III, or IV, prior to staining with PE-conjugated, pY694 phosphospecific Stat5 antibody. Histogram overlays show IL-2 stimulated samples (front) vs unstimulated controls (back).

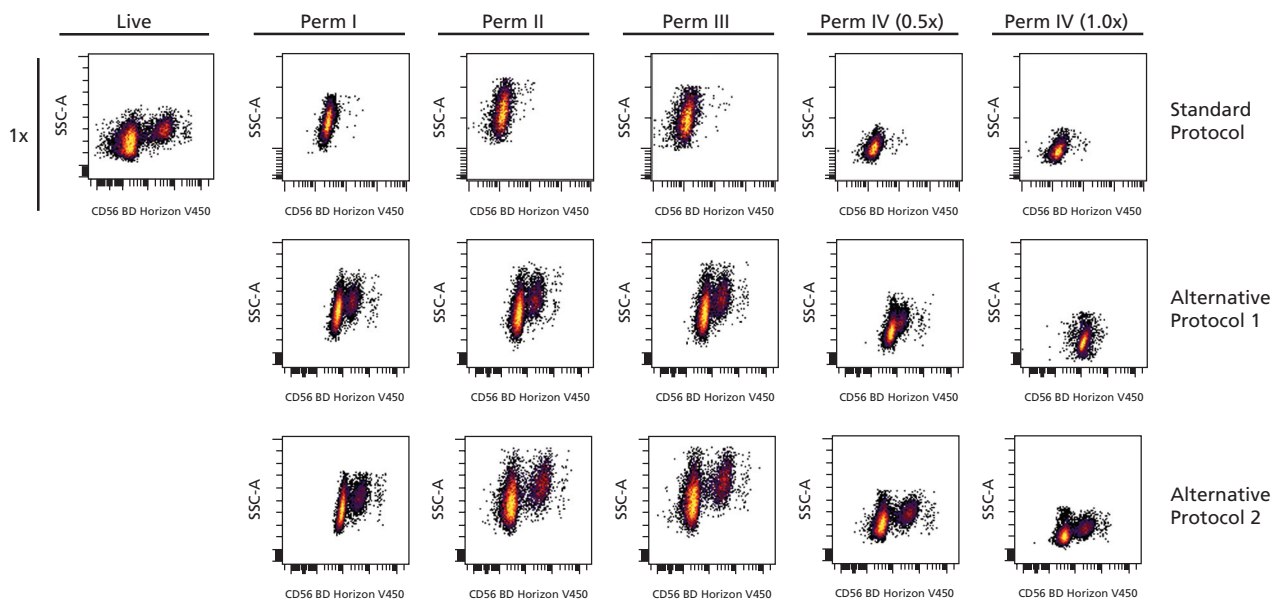


Figure 3. Surface marker expression can be impacted by the staining protocol used.

In this example, CD56 from human whole blood was stained after fixation and permeabilization (Standard Protocol), before permeabilization (Alternative Protocol 1), or before fixation (Alternative Protocol 2). In all three Fix/Perm protocols, cells were processed using BD Phosflow™ lyse/fix buffer, and BD Phosflow™ perm buffer I, II, III, or IV. BD FACS™ lysing solution was used for live cell staining.

Ordering Information

Description	Size	Cat.No.
BD Phosflow Perm/Wash Buffer I	125 mL	557885
BD Phosflow Perm Buffer II	125 mL	558052
BD Phosflow Perm Buffer III	125 mL	558050
BD Phosflow Perm Buffer IV	50 mL	560746
BD Cytotix Fixation Buffer	100 mL	554655
BD Phosflow Lyse/Fix Buffer	250 mL	558049



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