

Worksheet FC9

Digitonin Permeabilisation of Cells for Flow Cytometry

Note:

This method provides a general procedure for use with BZL01545. Specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

1. Prepare peripheral blood mononuclear cells and adjust to 1×10^6 /ml.
2. Aliquot 100 μ l into required number of tubes.
3. Stain with cell surface markers in usual way
4. Wash twice in ice-cold PBS. Decant supernatant.
5. Add 50 μ l PBS to each tube, followed by 100 μ l of 0.5% paraformaldehyde in PBS.
6. Incubate 20 minutes at room temperature.
7. Wash twice with PBS containing 0.05% Tween 20.
8. Decant supernatant. Add 100 μ l cold digitonin solution (10 μ g/ml in PBS).
9. Simultaneously add 10 μ l of test antibody at appropriate dilution.
10. Incubate 30 minutes.
11. Wash twice with PBS/Tween.
12. Resuspend in PBS, analyse by Flow Cytometry.

N.B. This procedure causes a reduction in FSC signal, and Flow Cytometer set up may need to be adjusted to compensate for this.

Buffers:

PBS/Paraformaldehyde Add 0.5 g paraformaldehyde to 100 ml PBS. Dissolve on heated stirrer and cool before use.

PBS/Tween: Add 50 μ l Tween 20 per 100 ml PBS.

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Digitonin: 10 µg/ ml in PBS.

PBS/BSA Phosphate Buffered saline pH 7.4 containing 20 mM glucose and 1% Bovine Serum Albumin.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE

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