JUN 21, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.q6rdzd6

External link:
<https://onlinelibrary.wiley.com/doi/abs/10.1002/cyto.a.10072>

Protocol Citation: Karsten Lücke 2018. FACS Staining of phosphorylated antigens.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.q6rdzd6>

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Protocol status: Working
 We use this protocol and it's working

Created: Jun 21, 2018

Last Modified: Jun 21, 2018

PROTOCOL integer ID:
 13233

FACS Staining of phosphorylated antigens

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ABSTRACT

This is a protocol for the detection of phosphorylated antigens via flow cytometry without the use of commercially available kits. It is adapted from the publication linked after the description

MATERIALS

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✕ Fetal Bovine Serum Gibco - Thermo Fischer Catalog #10270106

✕ Methanol J.T. Baker Catalog #9093-03

✕ Paraformaldehyde Sigma Aldrich Catalog #P6148

✕ Phorbol 12-myristate 13-acetate Sigma Aldrich Catalog #P8139

✕ Dulbecco's Phosphate Buffered Saline Sigma Aldrich Catalog #D5652

✕ Dimethyl sulfoxide (DMSO) Sigma Aldrich Catalog #D2650

✕ IMDM Gibco - Thermo Fischer Catalog #12440053

✕ Gentamicin Gibco - Thermo Fischer Catalog #15750037

✕ 2-Mercaptoethanol (50 mM) Gibco - Thermo Fischer Catalog #31350010

✕ L-Glutamine (200 mM) Gibco - Thermo Fischer Catalog #25030081

✕ Ionomycin from Streptomyces conglobatus Sigma Aldrich Catalog #I9657

✕ Albumin Bovine Fraction V Serva, Germany Catalog #11924

Keywords: phosflow, facs, staining

SAFETY WARNINGS

Wear gloves and safety goggles while handling 8%PFA and methanol.
Incubation with methanol only under a fume hood.

BEFORE START INSTRUCTIONS

Prepare IMDM (complete):

Add to the 500 ml IMDM Bottle:

25 ml fetal bovine serum (final concentration: 5%)

5 ml L-Glutamine (final concentration 2 mM)

500 µl Gentamicin (final concentration 50 µg/ml)

500 µl 2-Mercaptoethanol (final concentration 50 µM)

Prepare PBS with 5% BSA. Dilute part of it to 0,5%.

Prepare 1mM Ionomycin stocks in DMSO.

Prepare 10 µg/ml PMA Stocks in DMSO.

Prepare 8% PFA Solution in 1xPBS.

Cool methanol to 4 °C.

Prepare ice bath under the fume hood for incubation with methanol.

Cool down the centrifuge.

Incubation

- 1 Resuspend cell suspension at a concentration of 5×10^6 cells/ml. Incubate for 2 h at 37 °C and 5 %CO₂.

02:00:00 First
Incubation

10 mL IMDM (complete) per
Sample

37 °C and 5%
CO₂

- 2 Pipette 1 ml of single cell suspension in each of 6 FACS-Tubes. Label them accordingly (0 Minutes, 5 Minutes, 15 Minutes, 30 Minutes, 60 Minutes, 120 Minutes).

02:00:00 longest incubation

37 °C and 5% CO₂

- 3 Incubate the cells for the allotted time. Immediately after incubation take the samples out and add 200 µl 8% PFA (final concentration 1,5% PFA). Incubate at room temperature for 10 minutes.

00:10:00 for each sample

200 µL 8% PFA

20 °C

Permeabilization and Staining

- 4 Centrifuge for 5 minutes at 520 g and 4 °C. Decant supernatant. Resuspend samples in the return flow.

00:05:00 centrifugation

4 °C

- 5 Add 1 ml of ice cold methanol. Incubate for 30 minutes on ice.

1 mL Methanol per sample

00:30:00 Incubation on ice

4 °C on ice

- 6 Add 3 ml PBS/0,5% BSA to each sample. Centrifuge for 5 minutes at 520 g and 4 °C. Decant supernatant and Resuspend in 300 µl PBS/5% BSA.

300 µL PBS/5% BSA per sample

00:05:00 centrifugation

4 °C

3 mL PBS/0,5% BSA per sample

- 7 Transfer 75 µl of sample into a new FACS-Tube. Add 25 µl antibody mastermix.

Example for 10 stainings:

Antigen	Dye	Clone	Volume/S ample	Volume in mastermix
CD8a	PerCP	53-6.7	0.5	5

CD4	APC	RM4-5	0.5	5
TCRgd	FITC	eBioGL3	0.5	5
STAT3P	v450	4/P-STAT3	20	200
			PBS/5 % BSA	40

Incubate for 30 minutes at room temperature in the dark.

25 µL Mastermix per sample

20 °C

00:30:00 RT in the dark

- 8 Add 3 ml PBS/ 0.5% BSA. Centrifuge for 5 Minutes at 520 g and 4 °C. Decant supernatant.

3 mL PBS/0,5%
BSA

4 °C

00:05:00 centrifugation

- 9 Repeat Step 8. Add 200 µl of PBS/0,5% BSA for measuring.

go to step #8
undefined

200 µL PBS/0,5% BSA