FACS Staining of phosphorylated antigens

Karsten Lücke

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

Citation: Karsten Lücke FACS Staining of phosphorylated antigens. **protocols.io** dx.doi.org/10.17504/protocols.io.q6rdzd6
Published: 21 Jun 2018

Before start

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 concentraiton 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.

Materials

Fetal Bovine Serum 10270106 by Gibco - Thermo Fischer

Methanol 9093-03 by J.T. Baker

Paraformaldehyde P6148 by Sigma Aldrich

Phorbol 12-myristate 13-acetate P8139 by

Sigma Aldrich

Dulbecco's Phosphate Buffered Saline D5652 by Sigma Aldrich

Dimethyl sulfoxide (DMSO) D2650 by Sigma Aldrich

IMDM 12440053 by Gibco - Thermo Fischer

Gentamicin 15750037 by Gibco - Thermo Fischer

2-Mercaptoethanol (50 mM) 31350010 by Gibco - Thermo Fischer

L-Glutamine (200 mM) 25030081 by Gibco - Thermo Fischer

Ionomycin from Streptomyces conglobatus I9657 by Sigma Aldrich

Albumin Bovine Fraction V 11924 by Serva, Germany

Protocol

Incubation

Step 1.

Resuspend cell suspension at a concentration of $5*10^6$ cells/ml. Incubate for 2 h at 37 °C and 5 %CO_2.

AMOUNT

10 ml Additional info:
IMDM (complete) per
Sample

TEMPERATURE

37 °C Additional info: and
5% CO2

Incubation

Step 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).

TEMPERATURE

37 °C Additional info: and
5% CO2

Incubation

Step 3.

Incubate the cells for the alotted 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.

AMOUNT

200 µl Additional info: 8%
PFA

TEMPERATURE

20 °C Additional info:

Permeabilization and Staining

Step 4.

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

TEMPERATURE

4 °C Additional info:

Permeabilization and Staining

Step 5.

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

AMOUNT

1 ml Additional info:
Methanol per sample

TEMPERATURE

4 °C Additional info: on ice

Permeabilization and Staining

Step 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.

AMOUNT

300 µl Additional info:
PBS/5% BSA per sample

AMOUNT

3 ml Additional info:
PBS/0,5% BSA per sample

TEMPERATURE

4 °C Additional info:

Permeabilization and Staining

Step 7.

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

Example for 10 stainings:

Antigen	Dye	Clone	Volume/Sample	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.

AMOUNT

25 µl Additional info:
Mastermix per sample

TEMPERATURE

20 °C Additional info:

Permeabilization and Staining

Step 8.

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

AMOUNT

3 ml Additional info:
PBS/0,5% BSA

TEMPERATURE

4 °C Additional info:

Permeabilization and Staining

Step 9.

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

AMOUNT

200 µl Additional info:

PBS/0,5% BSA

GOTO

once -> go to step #8

Warnings

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