upstate
cell signaling solutions
a Serologicals Company

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Certificate of Analysis

Anti-FAK, clone 4.47, Alexa Fluor® 555 conjugate

(mouse monoclonal IgG_1)
Catalog # 16-234
Lot # 30488

Immunogen: GST fusion protein corresponding to residues 1-423 of human FAK (focal adhesion kinase). Clone 4.47.

Specificity: Recognizes and is specific for $p125^{FAK}$. Does not cross react with Pyk-2.

Species Cross-reactivity: Human, mouse, and rat.

Applications: Western blotting, immunofluorescence and flow cytometry.

Formulation: **100µg** Alexa Fluor® 555-conjugated protein G purified mouse IgG_1 in **200µl** of PBS containing 1% BSA, 0.05% Tween®-20, 0.05% sodium azide. Liquid at 4°C.

Storage and Stability: **Do Not Freeze.** Do not store the material diluted. Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

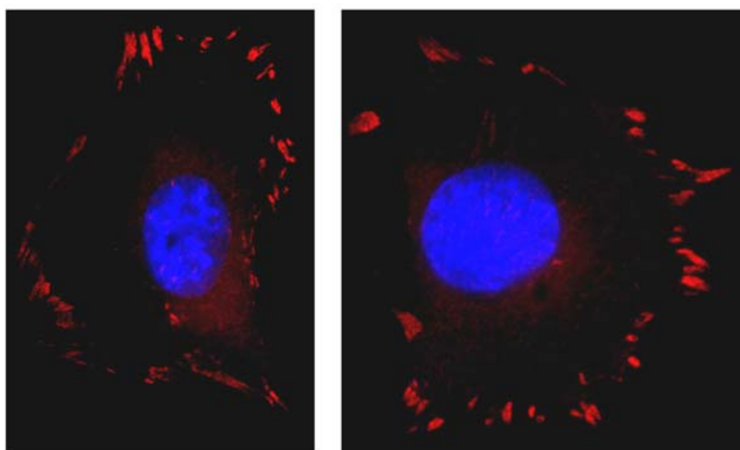
Quality Control Testing

Immunoblot Analysis: 2µg/ml of this lot detected FAK in non stimulated mouse 3T3/A31 cell lysates.

Immunocytochemistry: 2µg/ml of this lot showed positive immunostaining for FAK in 3T3 cells.

Flow Cytometry: 0.2µg of this lot detected FAK in fixed and permeabilized rat L6, Jurkat, and PC12 cells (see data, page two).

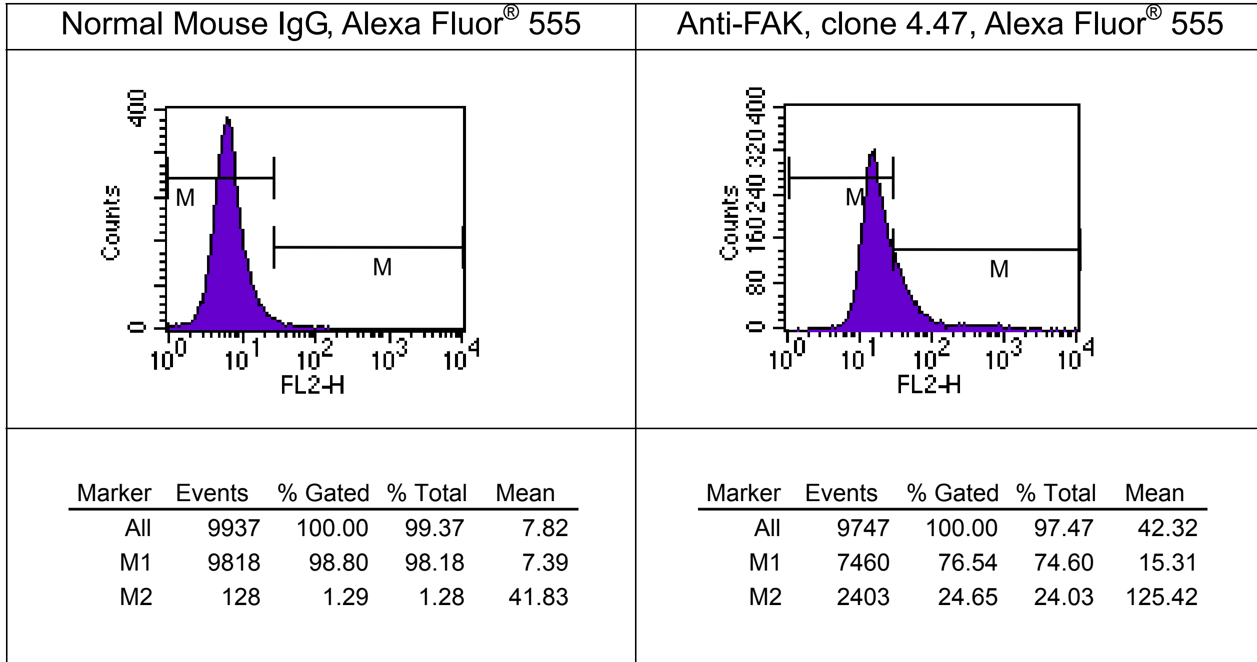
Included Negative Control: Catalog # 16-239, Alexa Fluor® 555-conjugated Normal Mouse IgG.



Immunocytochemistry
3T3 cells were stained with 2µg/ml of this lot of anti-FAK, clone 4.47, Alexa Fluor® 555 conjugate (red) and DAPI (blue).

Flow Cytometry Data

L6 cells



Normal Mouse IgG, Alexa Fluor® 555:

Marker	Events	% Gated	% Total	Mean
All	9937	100.00	99.37	7.82
M1	9818	98.80	98.18	7.39
M2	128	1.29	1.28	41.83

Anti-FAK, clone 4.47, Alexa Fluor® 555:

Marker	Events	% Gated	% Total	Mean
All	9747	100.00	97.47	42.32
M1	7460	76.54	74.60	15.31
M2	2403	24.65	24.03	125.42

Left panel: L6 cells stained with Normal Mouse IgG, Alexa Fluor® 555 (Catalog # 16-239) negative control.
Right panel: L6 cells stained with Anti-FAK, clone 4.47, Alexa Fluor® 555.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose.
2. Wet the blotted nitrocellulose in PBS for 5 minutes.
3. Block the blotted nitrocellulose in Odyssey® Blocking Buffer (Li-Cor®, Catalog # 927-40000) for 1 hour at room temperature with constant agitation.
4. Incubate the nitrocellulose with **2µg/ml of anti-FAK, clone 4.47, Alexa Fluor® 555 conjugate,** diluted in Odyssey® Blocking Buffer for 1 hour or longer with agitation at room temperature. Protect from light during incubation.
5. Wash the nitrocellulose 4 times for 5 minutes each at room temperature in PBS-0.05% Tween®-20 with agitation. Protect from light.
6. Rinse the nitrocellulose with PBS to remove residual Tween®-20. The membrane is now ready to scan.
7. Use detection method of choice (Amersham Biosciences Typhoon Imaging System).

Immunocytochemistry Protocol

1. Plate cells on coverslips in each well of a plate. Place the cells in a CO_2 incubator at 37ºC for 24 hours.
2. Remove media and wash the cells with PBS by rinsing 2 times.
3. Add fixative (3.7% formaldehyde) in PBS for 20 minutes at room temperature. Wash two times with PBS for 5 minutes.
4. Permeabilize with 0.5% Triton X-100 for 2 minutes.
5. Wash the cells 2 times with PBS for 5 minutes.
6. Incubate the cells with **2µg/ml of anti-FAK, clone 4.47, Alexa Fluor® 555 conjugate** in PBS for 1 hour.
7. Wash the cells 2 times with PBS for 5 minutes.
8. Mount the coverslip to a slide and dry.
9. Examine the cells under a fluorescent microscope.

Flow Cytometry Protocol

1. Harvest adherent cell lines via trypsinization or suspension cell lines by centrifugation. Count cells. You will need between 2×10^5 and 5×10^5 for each antibody test. Aliquot cells into 15ml conical test tubes.
2. Wash cells with 5ml PBS to remove culture media and serum. Pellet cells.
3. Fix cells by adding 500µl 3.7% paraformaldehyde in PBS at 4ºC for 20 minutes. Add 5ml PBS to cells. Invert to mix. Pellet cells.
4. Repeat 5ml PBS wash to remove remaining fixative.
5. Resuspend cells to 4×10^6 cells/ml in cold permeabilization buffer (0.5% Triton X-100 in PBS). The permeabilization technique given is a suggestion and should be optimized for individual applications and cell types for best results. Aliquot 50µl of cells (2.5×10^5 cells) per well of a 96 well round or V-bottom TC plate. Add 3.5µl of labeled antibody (60µg/ml antibody stock concentration = 0.21µg antibody per test well). Incubate on ice for one hour.
6. Add 150µl of PBS to each well. Centrifuge plate at 2000rpm for 5 minutes at 4ºC to pellet cells and remove supernatant to wash out unbound antibody.
7. Resuspend cell pellets in 150µl FACS buffer (4% FBS, 0.05% sodium azide, PBS). Transfer cell suspension to 1.2ml micro tube. Read on FACS machine using negative controls to set-up machine.
8. Number of cells per well and the amount of antibody used may need to be optimized for your individual application to give good, reproducible results.