FAK (Total) ELISA Kit

Catalog Number KHO0431 (96 tests)

Pub. No. MAN0005325 **Rev.** 2.0 (30)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ FAK (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of FAK (total) human, mouse, and rat in cell lysates. The assay will recognize both natural and recombinant FAK (total).

Focal Adhesion Kinase (FAK), also known as pp125FAK and FADK 1 (EC,2.7.1.112) is a non-receptor protein tyrosine kinase that localizes to focal adhesions. FAK appears to be ubiquitously expressed among all mammalian tissues, with highest expression levels observed in brain tissue.

FAK's amino-terminus, which bears homology with the band 4.1 family of proteins, plays a role in mediating interaction with the cell membrane, the cytoskeleton, and integrin proteins. FAK's carboxyl-terminus, which contains the focal adhesion targeting (FAT) domain, mediates interaction with focal adhesion associated proteins, including talin and paxillin. This kit can be used to normalize the phosphorylated FAK content of the samples when using the FAK [pY397] ELISA kit (Cat. # KHO0441).

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHO0431 (96 tests)
FAK (Total) Standard, lyophilized; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Plate, 96-well plate	1 plate
FAK (Total) Detection Antibody; contains 0.1% sodium azide; blue dye [1]	6.0 mL
Anti-Rabbit IgG HRP (100X); contains 3.3 mM thymol	0.125 mL
HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

[1] In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored Detection Antibody to help monitor the addition of solution to the reaction well. This does not in any way interfere with the test results.

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare Cell Extraction Buffer.

   Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
2. Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of FAK (total). Researchers must optimize the extraction procedures for their own applications.
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

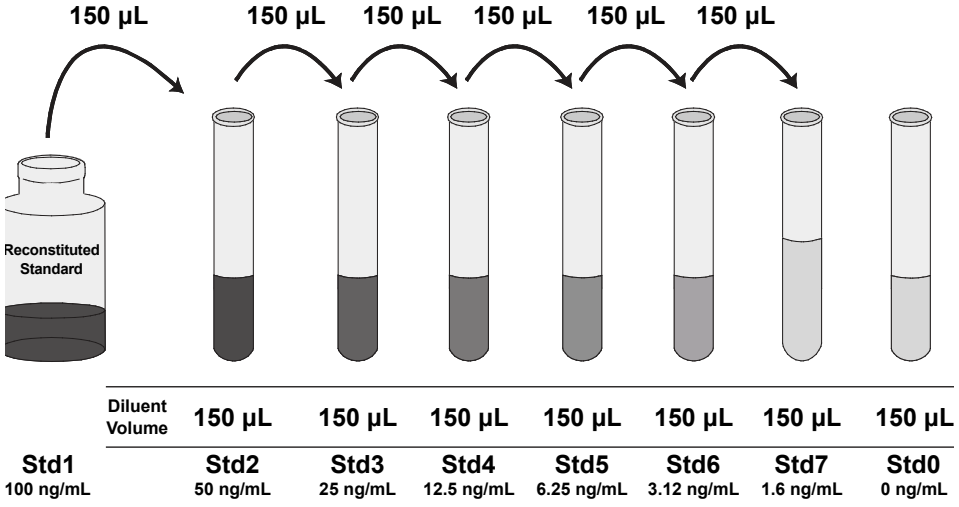
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:5 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 40 µL buffer). This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. SDS concentration should be less than 0.01% before adding to the plate. While a 1:5 sample dilution has been found to be satisfactory, higher dilutions such as 1:10 or 1:20 may be optimal.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This FAK (Total) Standard is prepared using purified, full length, recombinant FAK protein expressed in Sf21 cells.

1. Reconstitute FAK (Total) Standard to 100 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 100 ng/mL FAK (total). **Use the standard within 1 hour of reconstitution.**
2. Add 150 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.12, 1.6, and 0 ng/mL FAK (total).
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 **Bind antigen and add detector**

a. Add 50 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 50 µL of FAK (Total) Detection Antibody solution into each well except the chromogen blanks.
c. Cover the plate with a plate cover and incubate 3 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 **Add IgG HRP**

a. Add 100 µL 1X Anti-Rabbit IgG HRP Solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 **Add Stabilized Chromogen**

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 **Add Stop Solution**

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 100 ng/mL FAK (total).

Standard FAK (Total) (ng/mL)	Optical Density (450 nm)
100	3.26
50	1.86
25	0.99
12.5	0.60
6.25	0.40
3.12	0.29
1.6	0.18
0	0.15

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	47.1	24.6	9.8
Standard Deviation	2.4	1.7	0.99
% Coefficient of Variation	5.1	6.9	10.1

Intra-assay precision

Samples of known FAK (total) concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	47.2	24.9	10.1
Standard Deviation	2.0	0.82	0.63
% Coefficient of Variation	4.2	3.3	6.2

High-dose hook effect

Samples spiked with FAK (total) Standard up to 400 ng/mL give responses higher than that obtained from the last standard point.

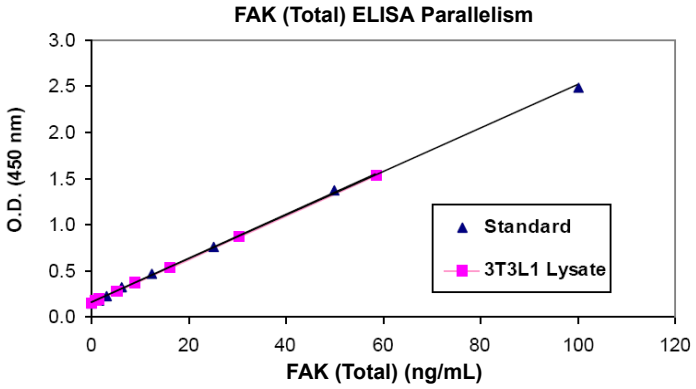
Linearity of dilution

3T3L1 cells were grown in cell culture medium containing 10% fetal calf serum and lysed with cell extraction buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for FAK (total). Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Lysate		
	Measured (ng/mL)	Expected	
		(ng/mL)	%
Neat	34.56	34.56	100
1/2	17.13	17.28	100.8
1/4	8.44	8.64	102.4
1/8	4.17	4.32	103.6
1/16	2.53	2.16	85.4

Parallelism

Natural FAK from 3T3L1 cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the FAK (total) standard curve. The standard accurately reflects FAK (total) content in samples.



Recovery

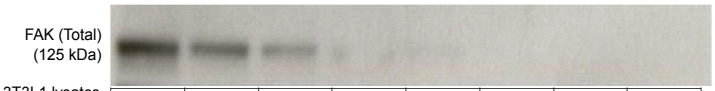
To evaluate recovery, FAK (Total) Standard was spiked at 3 different concentrations into 20% Cell Extraction Buffer. The average recovery was 101%.

Sensitivity

The analytical sensitivity of this assay is <1.6 ng/mL of FAK (total). This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 16 times. Using 3T3L1 cells, this level of sensitivity was equivalent to the detection of FAK in 6,000 cells.

The sensitivity of this ELISA was compared to western blotting using known quantities of FAK. The data presented in the following figure show that the sensitivity of the ELISA is approximately 2X greater than that of western blotting. The bands shown in the western blotting data were developed using rabbit anti-FAK, and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

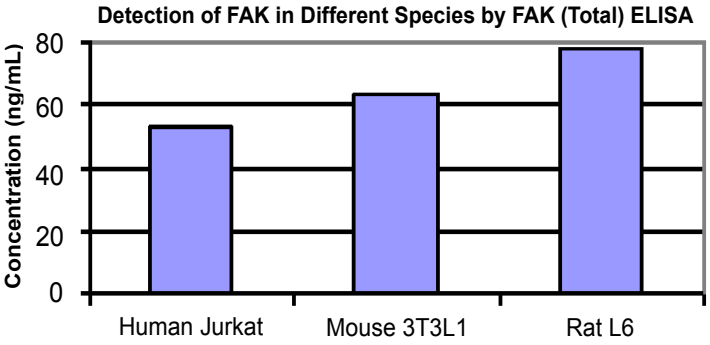
Figure 1: Detection of FAK (Total) by ELISA vs Western Blot:



3T3L1 lysates (µg/test)	20	10	5	2.5	1.25	0.63	0.32	0
ELISA: OD 450 nm	3.26	1.86	0.99	0.60	0.40	0.29	0.18	0.15

Specificity

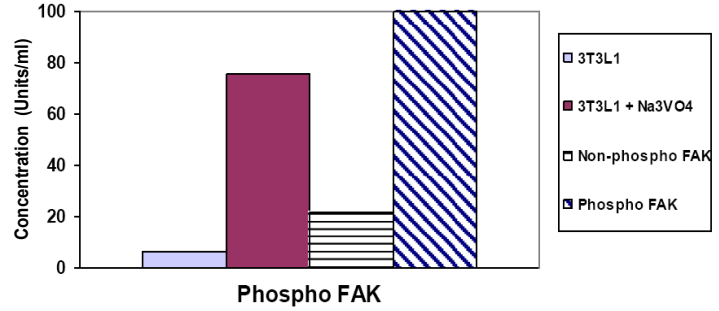
Two hundred µg/mL of cell extracts from Jurkat, 3T3L1 and L6 cells were analyzed by FAK (Total) ELISA Kit.

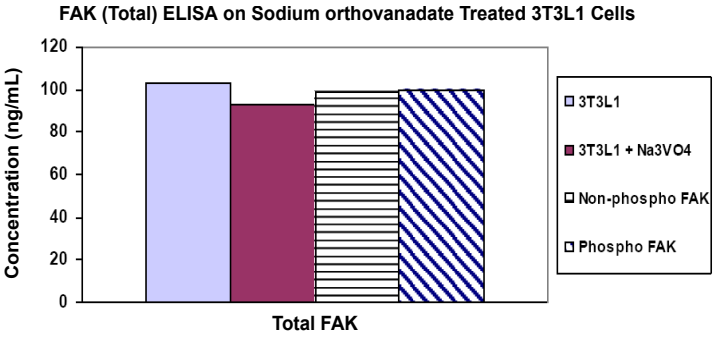


The FAK (Total) ELISA Kit is specific for measurement of total FAK protein. The following proteins were tested in the assay and found to have no cross-reactivity: p38 MAPK, AKT, EGFR, GSK 3beta, JNK, HSP27, Paxillin, and SRC.

In the following two figures, 3T3L1 cells were treated with 1 mM sodium orthovanadate for 5 hours and untreated 3T3L1 cells were used as control. Cell extracts were prepared in 0.1% SDS extraction buffer. 50 µL of each cell lysate (400 µg/mL) was analyzed with FAK (Total) ELISA Kit and FAK [pY397] ELISA (Cat. # KHO0441). The FAK (total) detected phosphorylated FAK recombinant protein and phosphorylated FAK in orthovanadate-treated 3T3L1 cells, but not the non-phosphorylated FAK recombinant protein or non-phosphorylated FAK in untreated 3T3L1 cells (Figure 4). In contrast, FAK (total) detected both phosphorylated and non-phosphorylated FAK recombinant protein and FAK in orthovanadate-treated cells and untreated control (see the last figure below).

Figure 4: FAK[pY397] ELISA on Sodium orthovanadate Treated 3T3L1 Cells





This "Total" assay is designed to allow normalization of FAK content among samples to permit interpretation of results from the phosphorylation site-specific FAK [pY397] ELISA Kit available from (Cat. # KHO0441).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.