Human Smad2 [pSpS465/467] ELISA Kit

Catalog Number KHO8011 (96 tests)

Pub. No. MAN0015804 **Rev.** 3.0 (31)

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™ Human Smad2[pSpS465/467] ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of Smad2 [pSpS465/467] in cell lysates or tissue extract.

Smad proteins transduce signals from transforming growth factor-β (TGF-β) superfamily ligands that regulate cell proliferation, differentiation, matrix production and apoptosis through activation of type I receptor serine/threonine kinases. Smad2, one of the key components in TGF-β pathway, functions by carrying signals from the cell surface directly to the nucleus. Smad2 mediates TGF-β signaling to regulate cell growth and differentiation. In response to TGF-β, Smad2 is phosphorylated on Ser 465/467 by activin type 1 receptor kinases

Contents and storage

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

Components	Cat. No. KHO8011 (96 tests)
Antibody Coated Wells. 96-well plate	1 plate
Smad2 [pSpS465/467] Detection Antibody; contains 0.1% sodium azide	11 mL
Smad2 [pSpS465/467] Standard, Lyophilized; Contains 0.1% sodium azide	2 vials
Wash Buffer Concentrate (25X)	100 mL
Standard Diluent Buffer; Contains 0.1% sodium azide	25 mL
Anti-Rabbit IgG HRP (100X)	0.125 mL
HRP Diluent. Contains 3.3 mM thymol	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	3

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 100 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

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 human smad2 [pSpS465/467]. 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

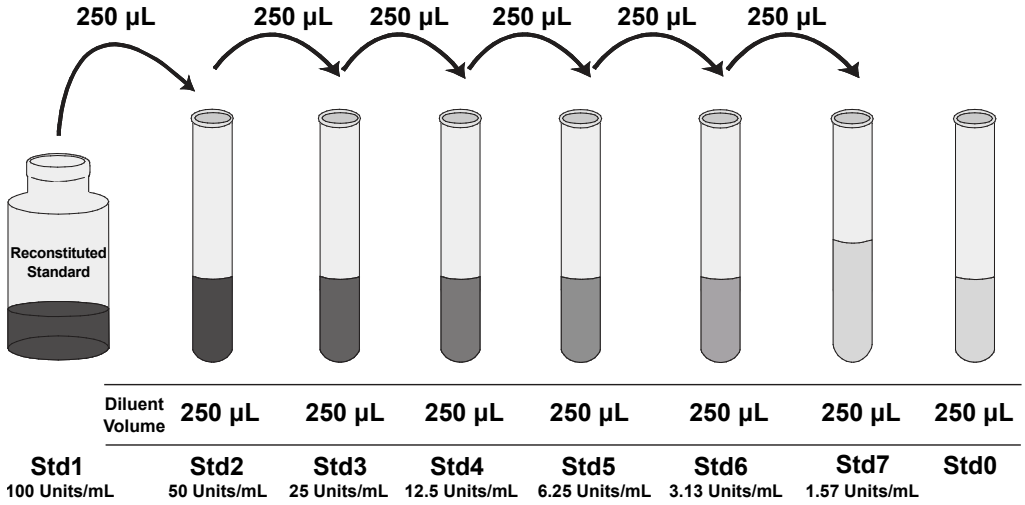
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:10 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 90 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 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute the Smad2 [pSp465/467] Standard to 100 units/ 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 units/ mL Hu Smad2 [pSpS465/467]. **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.13, 1.57, and 0 units/ mL.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

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

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) 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**

a. Add 100 µL of Standard or cell/tissue extract (>1:10) to appropriate wells. Leave the wells for chromogen blanks empty. Tap gently on the side of the plate to mix.
b. Cover the plate with a plate cover and incubate 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 **Add detector antibody**

a. Add 100 µL of Smad2 [pSpS465/467] Detection Antibody solution into each well except the chromogen blanks. Tap gently on the side of the plate to mix.
b. Cover the plate with a plate cover and incubate 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 **Add IgG HRP**

a. Add 100 µL Anti-Rabbit IgG HRP 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.

4 **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.

5 **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 was obtained for the various obtained for the various standards over the range of 0-10 units/ mL of Human Smad2 [pSpS465/467].

Standard Hu Smad2 [pSpS465/467] (units/mL)	Optical Density (450 nm)
100	3.230
50	2.413
25	1.566
12.5	0.907
6.25	0.548
3.13	0.342
1.57	0.228
0	0.111

Intra-assay precision

Samples of known human smad2 [pSpS465/467] concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (Units/mL)	48.42	12.05	2.70
Standard Deviation	1.52	0.27	0.16
%Coefficient of Variation	3.15	2.29	6.15

Inter-assay precision

Samples were assayed 48 times in 3 assays (16 points per assay) to determine precision between assays.

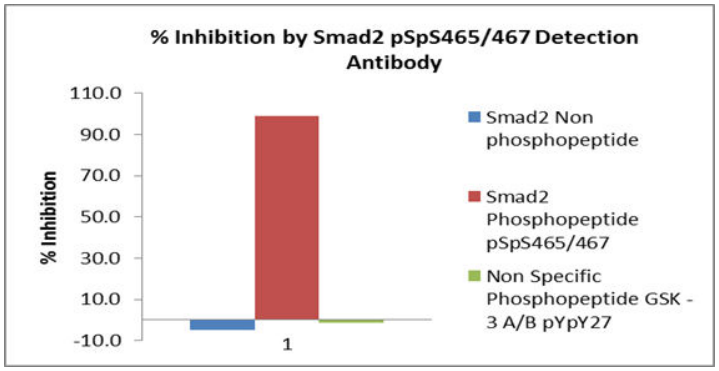
Parameters	Sample 1	Sample 2	Sample 3
Mean (Units/mL)	46.08	11.53	2.85
Standard Deviation	2.76	0.61	0.24
%Coefficient of Variation	6.00	5.32	8.37

Specificity

The peptide blocking competition data presented show that only the phosphopeptide containing the phosphorylated serine at site 465 and 467 could block the ELISA signal. The non-phosphorylated peptide sequence or other phosphopeptides did not block the signal.

Table 1

	Smad2 Non-Phosphopeptide	Smad2 Phosphopeptide pSpS465/467	Non-specific Phosphopeptide GSK-3 A/B pY279/216
Standard 0	0.100	0.099	0.101
Standard 1	2.913	2.865	2.884
Peptide Blocking	2.974	0.132	2.896
	3.083	0.120	2.946



Sensitivity

The analytical sensitivity of the assay is <0.42 units/mL Hu Smad2 [pSpS465/467]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 32 times, and calculating the corresponding concentration.

Recovery

To evaluate recovery, Smad2 [pSpS465/467] Standard was spiked at 3 different concentrations into 10% Cell Extraction Buffer. The percent recovery was calculated as an average of 100%.

Limited product warranty

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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: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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