GSK-3ß [pS9] ELISA Kit

Catalog Number KHO0461 (96 tests)

Pub. No. MAN0014926 **Rev.** 2.0

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 GSK-3β [pS9] ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of GSK-3β [pS9] in lysates from human, mouse, and rat cells. The assay recognizes both natural and recombinant GSK-3β [pS9].

Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine protein kinase, and one of two mammalian GSK-3 isoforms. The two isoforms, designated GSK-3α (Mr=51 kDa) and GSK-3β (Mr=) share 95% homology in their kinase domains. GSK-3β homologs are ubiquitously expressed in eukaryotes.

For normalizing the GSK-3β [pS9] content of the samples, a GSK-3β (Total) ELISA Kit (Cat. No. KHO0451) is available for detection of GSK-3β content independent of phosphorylation status.

Contents and storage

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

Contents	Cat. No. KHO0461 (96 tests)
GSK-3ß [pS9] Standard; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
GSK-3ß [pS9] Antibody Coated Wells, 96-well strip-well plate	1 plate
GSK-3ß [pS9] Detection Antibody; contains 0.1% sodium azide; blue dye [1]	6 mL
Anti-Rabbit IgG HRP (100X); contains 3.3 mM thymol	0.125 mL
HRP Diluent; contains 3.3 mM thymol; yellow dye [1]	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 Standard Diluent Buffer, Detection Antibody, and HRP Diluent 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
- 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 5 mL of 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 1 mM PMSF (0.3 M stock in DMSO) and 250 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714).

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 GSK-3β [pS9]. [FOR EXAMPLE, 5×10^7 Jurkat cells can be extracted in 1 mL of Cell Extraction Buffer. 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. While a 1:5 sample dilution has been found to be satisfactory, higher dilutions such as 1:10 or 1:20 may be optimal.

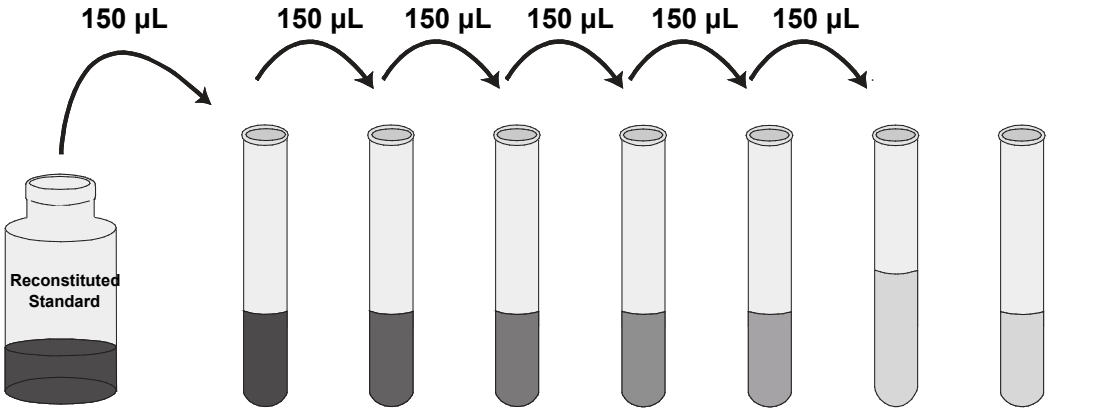
  For 5×10^7 Jurkat cells, use 1– 10µL of the clarified lysate diluted to 50 µL in Standard Diluent Buffer for each well.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This GSK-3β [pS9] Standard is prepared using purified, full length, recombinant, phosphorylated GSK-3β protein expressed in s*f*21 cells. One unit of standard is equivalent to the amount of GSK-3β [pS9] phosphorylated from 300 pg of total GSK-3β protein.

1. Reconstitute GSK-3β [pS9] 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 GSK-3β [pS9]. **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 Units/mL GSK-3β [pS9].
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 GSK-3β [pS9] Detection Antibody solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. 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 Units/mL GSK-3β [pS9].

Standard GSK-3β [pS9] (Units/mL)	Optical Density (450 nm)
100	2.72
50	1.77
25	1.04
12.5	0.68
6.25	0.39
3.12	0.28
1.6	0.20
0	0.15

Inter-assay precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (Units/mL)	62.79	12.66	4.90
Standard Deviation	4.44	1.21	0.35
% Coefficient of Variation	7.06	9.58	7.05

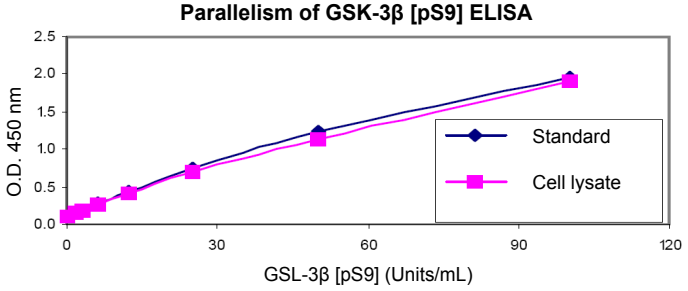
Intra-assay precision

Samples of known GSK-3β [pS9] concentrations were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (Units/mL)	59.04	12.44	4.85
Standard Deviation	3.41	0.80	0.33
% Coefficient of Variation	5.77	6.46	6.80

Parallelism

Natural GSK-3β [pS9] from LiCl-treated Jurkat cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the GSK-3β [pS9] standard curve. The standard accurately reflects GSK-3β [pS9] content in samples.



High-dose hook effect

Samples spiked with GSK-3β [pS9] Standard up to 200 Units/mL give responses higher than that obtained from the highest standard point.

Linearity of dilution

Lysate from Jurkat cells treated with LiCl (100 mM for 3 hours) and prepared in Cell Extraction Buffer was diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Measured (Units/mL)	Expected	
		(Units/mL)	%
Neat	33.2	33.2	100
1/2	17.1	16.6	103
1/4	8.8	8.3	106
1/8	3.9	4.2	93
1/16	1.9	2.1	90

Recovery

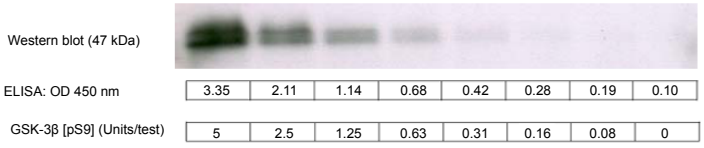
GSK-3β [pS9] Standard was spiked at 3 different concentrations into 50 µg/mL Jurkat cell lysate to evaluate recovery. The average recovery was 98.9%.

Sensitivity

The analytical sensitivity of this assay is <0.4 Units/mL GSK-3β [pS9]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. The value corresponds to the amount of GSK-3β extracted from 5,000 Jurkat cells cultured under optimal conditions.

In addition, the sensitivity of the ELISA is ~2-fold greater than that of western blot when tested against known quantities of GSK-3β.

Detection of GSK-3β [pS9] by ELISA vs western blot

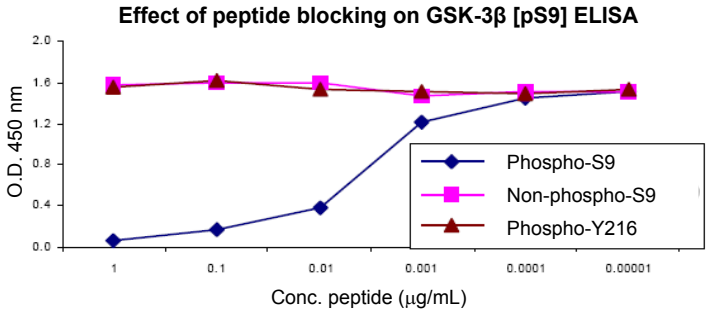


ELISA: OD 450 nm	3.35	2.11	1.14	0.68	0.42	0.28	0.19	0.10
GSK-3β [pS9] (Units/test)	5	2.5	1.25	0.63	0.31	0.16	0.08	0

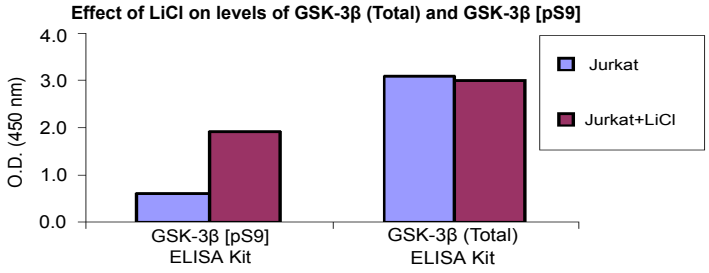
Specificity

The GSK-3β [pS9] ELISA Kit recognizes GSK-3β [pS9] protein from human, rat, and mouse. Other species have not been tested. The assay is specific for GSK-3β phosphorylated at serine residue 9, and does not cross-react with GSK-3α phosphorylated at serine residue 21.

Specificity for GSK-3β [pS9] was confirmed by peptide competition. The data shows that only the phosphopeptide containing the phosphorylated serine 9 blocks the ELISA signal. The non-phosphorylated peptide sequence or other phosphopeptides from the GSK-3β sequence did not block the signal.



Specificity for GSK-3β [pS9] was also confirmed by comparing results with the GSK-3β (Total) ELISA Kit (Cat. No. KHO0451). Jurkat lysates from cells treated with 100 mM lithium chloride were compared to untreated controls. The results show that the total GSK-3β content remained constant between treated and untreated samples, while the amount of detected GSK-3β [pS9] increased in LiCl treated samples, .



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's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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