PHOSPHO GSK-3β ELISA Kit

Catalog Number: EMS2GSK3P

Product description

An immunoassay for the quantitative determination of phosphorylated glycogen synthase kinase 3β (pGSK-3β) in cell lysates.

Contents and storage

Upon receipt, store the kit at ≤ –20°C. Avoid repeated freeze-thaw cycles.

Description	Size
Antibody coated 96-well plate	1 plate
Reagent Diluent	100 mL
Phospho GSK-3β Detection Antibody	10 mL
Phospho GSK-3β Conjugate	10 mL
Lysis Buffer	100 mL
GSK-3β Standard (100,000 pg/mL recombinant Phospho GSK-3β)	0.1 mL
20X Wash Buffer	100 mL
TMB Substrate Solution	10 mL
Stop Solution (1N HCl)	10 mL
Plate Sealer	3 each

Additional required materials

- Deionized or distilled water
- Phenylmethylsulfonyl fluoride (PMSF)
- Protease Inhibitor Cocktail (PIC)
- Sodium orthovanadate
- Sodium pyrophosphate
- Precision pipettes (for volumes between 100 µL and 1,000 µL)
- Repeater pipettes (for dispensing 100 µL)
- Disposable beaker for diluting buffer concentrates
- 12 × 75 mm polypropylene tubes
- Graduated cylinders
- Microplate shaker
- Absorbent lint free paper for blotting
- Microplate reader capable of reading at 405 nm
- Software for interpolating sample values from optical density readings utilizing a four parameter logistic curve fit

General guidelines

- Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard or reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

Assay compatibility

The Phospho GSK-3β ELISA is compatible with pGSK-3β samples in a wide range of matrices after dilution in Reagent Diluent Plus Inhibitors.

Prepare Lysis Buffer Plus Inhibitors

Prepare fresh Lysis Buffer Plus Inhibitors each time cells are lysed by adding inhibitors to Lysis Buffer at the final concentration listed in the following table.

Inhibitor	Final concentration
Protease Inhibitor Cocktail (PIC)	0.5 µL/mL
PMSF	1 mM
Sodium orthovanadate	2 mM
Sodium pyrophosphate	20 mM

Prepare Reagent Diluent Plus Inhibitors

Use the Reagent Diluent Plus Inhibitors for all Phospho GSK-3β Standard and sample dilutions to ensure optimal integrity of pGSK-3β.

Prepare fresh Reagent Diluent Plus Inhibitors for each assay by adding inhibitors to Reagent Diluent at the final concentration listed in the following table.

Inhibitor	Final concentration
PMSF	1 mM
Protease Inhibitor Cocktail (PIC)	0.5 µL/mL

Prepare 1X Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 50 mL of 20X Wash Buffer with 950 mL of deionized or distilled water. Label as 1X Wash Buffer.

   The diluted buffer is stable for up to 3 months at room temperature.

Sample handling

- Store samples at –70°C to avoid loss of bioactive pGSK-3β.
- Avoid excessive freeze-thaw cycles.
- Slowly warm frozen samples to 2°C to 8°C and centrifuge, if necessary, to isolate residual debris.
- Samples containing rabbit serum or rabbit IgG are not suitable for use in this assay.

Sample preparation guidelines

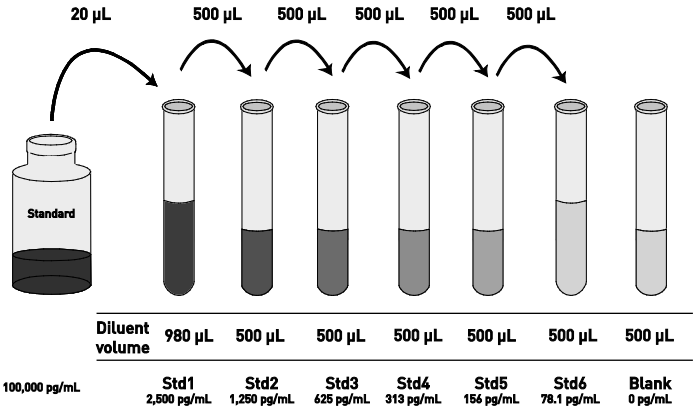
- Lyse samples with fresh Lysis Buffer Plus Inhibitors immediately before use.
- Dilute samples lysed in fresh Lysis Buffer Plus Inhibitors at least 1:16 with Reagent Diluent Plus Inhibitors prior to assay.
- Samples diluted sufficiently into fresh Reagent Diluent Plus Inhibitors can be read directly from a standard curve.
- If using other lysis buffers, determination of an appropriate dilution for samples and assay validation is required.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Standard preparation guidelines

- Only standard curves generated in fresh Reagent Diluent Plus Inhibitors should be used to calculate the concentration of pGSK-3β.
- Use the diluted standards within 60 minutes.

Dilute standards

1. Allow the 100,000 pg/mL GSK-3β standard to warm to room temperature.
2. Label six polypropylene tubes #1 through #6.
3. Add 980 µL Reagent Diluent Plus Inhibitors to Tube #1.
4. Add 500 µL Reagent Diluent Plus Inhibitors to Tubes #2 to #6.
5. Add 20 µL of the GSK-3β standard to Tube #1.
6. Make serial dilutions of the standard as shown in the diagram below.



ELISA procedure

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 to 8°C for future use. Allow kit components to come to room temperature for at least 30 minutes before use.

Run all standards and samples in duplicate.

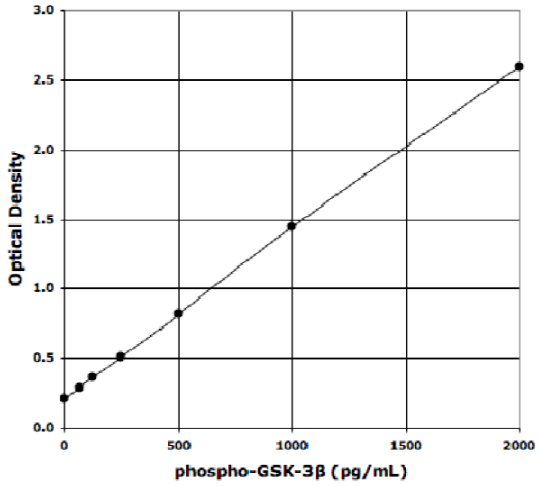
1. Add 100 µL of Reagent Diluent Plus Inhibitors into the S0 wells (0 pg/mL Standard).
2. Add 100 µL of Standards #1 through #6 into the appropriate wells.
3. Add 100 µL of the Samples into the appropriate wells.
4. Tap the plate gently to mix the contents.
5. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 1 hour.
6. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes.
7. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Add 100 µL of the yellow Phospho GSK-3β Detection Antibody into each well, except the Blank well.
9. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 1 hour.
10. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes.
11. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
12. Add 100 µL of the blue Phospho GSK-3β Conjugate into each well, except the Blank well.
13. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
14. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes.
15. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
16. Add 100 µL of the TMB Substrate Solution to every well. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
17. Add 100 µL of Stop Solution to every well and read the plate immediately.
18. Blank the plate reader against the Blank wells, read the optical density at 405 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculations

Several options are available for the calculation of the concentration of pGSK-3β in the samples. It is recommended that the data be analyzed by a 4 parameter logistic curve fitting program. If data reduction software is not available, the concentration of the pGSK-3β can be determined by calculating the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample. Using linear graph paper, plot the average Net OD for each standard versus phospho-GSK-3β concentration in each standard. Approximate a straight line through the points. The concentration of pGSK-3β in the unknowns can be determined by interpolation. Samples outside of the standard curve range will need to be reanalyzed using a different dilution.

Typical standard curve

A typical standard curve is shown below. This curve must not be used to calculate pGSK-3β concentrations; a standard curve must be run with every assay.



Performance characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols.

Sensitivity

The sensitivity or limit of detection of the assay is 9 pg/mL. The sensitivity was determined by interpolation at two standard deviations above the mean signal at background (0 pg/mL) using data from six standard curves.

Linearity

A sample containing 2,000 pg/mL pGSK-3β was serially diluted in Reagent Diluent over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a line with a slope of 0.0012 and correlation coefficient of 0.9997.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of pGSK-3β and running these samples multiple times (n=20) in the same assay. Inter-assay precision was determined by measuring 3 samples with low, medium and high concentrations of pGSK-3β in multiple assays over 3 days (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of pGSK-3β determined in these assays as calculated by a 4-parameter logistic curve-fitting program.

Intra-assay	pGSK-3β (pg/mL)	%CV
Low	110	4.8
Medium	323	4.1
High	903	2.0

Inter-assay	pGSK-3β (pg/mL)	%CV
Low	108	6.0
Medium	311	7.0
High	891	9.6

Cross-reactivity

The Phospho GSK-3β 1/2 ELISA Kit is specific for bioactive pGSK-3β.

Compound	Cross Reactivity
Phospho-GSK-3β	100%
GSK-3α, active	5.4%
β-catenin, p21, ATP Citrate Lyase, MEK1, AKT1, phospho-JNK1, phospho-ERK2, ERK2	<0.3%

Sample recovery

pGSK-3β concentrations were measured in RIPA Cell lysates diluted in Lysis Buffer Plus Inhibitors and measured in the kit.

Sample	% Recovery
25.5 µg/mL total protein	138%
12.75 µg/mL total protein	102%
6.38 µg/mL total protein	101%
3.19 µg/mL total protein	100%

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

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