ERK1/2 [pThr^{202}/Tyr^{204}] ELISA Kit

Catalog Number: EMS2ERKP

Product description

An immunoassay for the quantitative determination of ERK1/2 dual-phosphorylated at threonine 202 and tyrosine 204 in cell lysates.

Contents and storage

The kit and components are shipped at -20°C. Upon receipt, store the ERK1/2 [pThr^{202}/Tyr^{204}] ELISA Kit at -20°C.

Description	Amount
Antibody coated 96-well plate	1 plate
Reagent Diluent Concentrate	100 mL
ERK1/2 [pThr^{202}/Tyr^{204}] Detection Antibody, yellow*	10 mL
ERK1/2 [pThr^{202}/Tyr^{204}] Standard (1,000 pg recombinant phospho-ERK, lyophilized)	2 vials
Goat anti-rabbit-HRP Conjugate, blue*	10 mL
Cell Lysis Buffer	100 mL
20X Wash Buffer	100 mL
TMB Substrate	10 mL
Stop Solution (1N HCl)	10 mL
Plate Sealer	3 each

*Some components are colored yellow or blue for ease of use.

Additional required materials

- Deionized or distilled water
- Phenylmethylsulfonyl fluoride (PMSF)
- Protease Inhibitor Cocktail (PIC) (Sigma #P8340 or equivalent)
- Sodium pyrophosphate
- Sodium orthovanadate
- 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 450 nm, preferably with correction between 570 and 590 nm.

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.
- Allow kit components to come to room temperature for at least 30 minutes before use.
- 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 ERK1/2 [pThr^{202}/Tyr^{204}] ELISA Kit is compatible with phosphorylated ERK samples in a wide range of cell lysates and buffers after dilution in Reagent Diluent Plus Inhibitors.

Reagent Preparation

Cell Lysis Buffer Plus Inhibitors

Allow to come to room temperature. Ensure Cell Lysis Buffer is completely in solution prior to use. Immediately prior to cell lysis, add protease inhibitors (PMSF and PIC) and phosphatase inhibitors (sodium orthovanadate and sodium pyrophosphate to the buffer. Add PMSF to a final concentration of 1 mM. Add sodium orthovanadate to a final concentration of 2 mM. Add sodium pyrophosphate to a final concentration of 20 mM.

Fresh Cell Lysis Buffer Plus Inhibitors must be made each time the cells are lysed.

Reagent Diluent Plus Inhibitors

Immediately prior to use in the assay, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL or equivalent concentration according to alternate vendor's specification sheet. Add PMSF to a final concentration of 1 mM.

This modified Reagent Diluent must be used for standard reconstitution and all sample and standard dilutions to ensure optimal integrity of pERK. Fresh Reagent Diluent Plus Inhibitors must be made for each assay.

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 Wash Buffer Concentrate 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 pERK.
- Avoid excessive freeze-thaw cycles.
- Slowly warm frozen samples to 2°C to 8°C and mix gently prior to assay.

Sample preparation guidelines

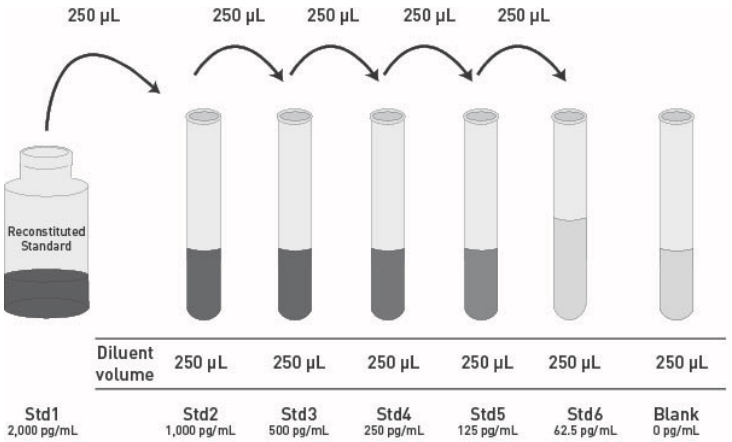
- Samples lysed with Cell Lysis Buffer Plus Inhibitors require further dilution with Reagent Diluent Plus Inhibitors prior to running the assay.
- A minimum 1:8 dilution is recommended to remove matrix interference in the assay.
- Samples diluted sufficiently into Reagent Diluent Plus Inhibitors can 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 ERK1/2 [pThr^{202}/Tyr^{204}].
- Use the diluted standards within 60 minutes of preparation. Allow to warm to room temperature before use.

Reconstitute and dilute standards

1. Add 500 µL Reagent Diluent Plus Inhibitors to the lyophilized ERK1/2 [pThr^{202}/Tyr^{204}] Standard vial and vortex. Label as 2,000 pg/mL pERK.
2. Wait for 5 minutes and vortex again prior to use.
3. Add 250 µL reconstituted standard to one tube containing 250 µL of Reagent Diluent Plus Inhibitors. Label as 1,000 pg/mL pERK.
4. Add 250 µL of Reagent Diluent Plus Inhibitors to each of 5 tubes labeled as: 500, 250, 125; 62.5, and 0 pg/mL pERK.
5. Discard all remaining reconstituted and diluted standards after completing assay.



ELISA procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening. 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.

Run all standards and samples in duplicate.

1. Add 100 µL of standards into the appropriate wells.
2. Add 100 µL of the samples into the appropriate wells.
3. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 1 hour.
4. 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.
5. 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.
6. Add 100 µL of the yellow ERK1/2 [pThr^{202}/Tyr^{204}] Detection Antibody into each well, except the Blank well.
7. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 1 hour.
8. 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.
9. 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.
10. Add 100 µL of the blue Goat anti-rabbit-HRP Conjugate into each well, except the Blank well.
11. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
12. 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. 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.
13. Add 100 µL of the TMB Substrate Solution to every well. Incubate at room temperature on a plate shaker (~500 rpm) for 30 minutes.
14. Add 100 µL of Stop Solution to every well and read the plate immediately.
15. Blank the plate reader against the Blank wells and read the optical density at 450 nm, preferably with correction between 570 and 590 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 ERK1/2 [pThr^{202}/Tyr^{204}] 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 ERK1/2 [pThr^{202}/Tyr^{204}] can be calculated as follows:

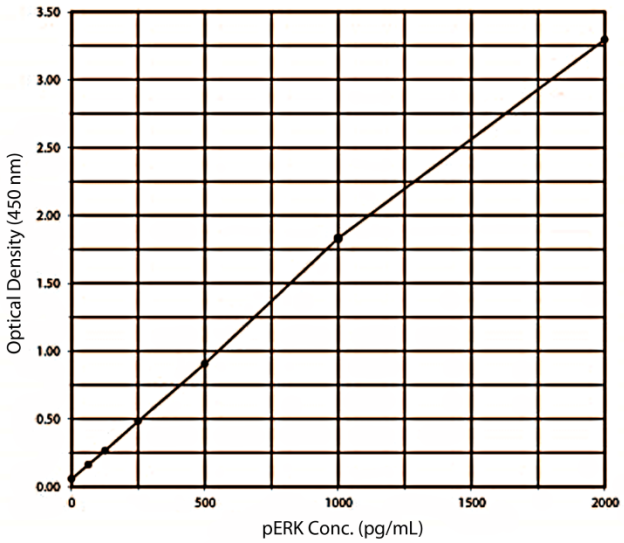
1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD
2. Plot the Average Net OD for each standard versus ERK1/2 [pThr^{202}/Tyr^{204}] concentration in each standard. Approximate a straight line through the points. The concentration of ERK1/2 [pThr^{202}/Tyr^{204}] in the unknowns can be determined by interpolation.

Note: Samples measured in the phospho-ERK ELISA can be expressed in terms of concentration by weight or activity. When reconstituted according to direction, the standard stock concentration is 2000 pg/mL. To convert this value to Units/mL, the weight concentration is multiplied by the specific activity of the standard. The specific activity of the standard is ~10,000,000 Units/mg where one Unit of pERK activity is equal to 1 pmole phosphate incorporated into 100 µM myelin basic protein per minute at 30°C in a total reaction volume of 30 µL.

Typical standard curve

A typical standard curve is shown below. This curve must not be used to calculate pERK 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 analytical sensitivity of pERK is 2.67 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained from the average OD bound for 24 wells run as 0 pg/mL Standard, compared to the average OD for 24 wells run with 2,000 pg/mL Standard. The detection limit was determined as the concentration of pERK measured at two (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Linearity

A sample containing 1,400 pg/mL pERK was serially diluted 5 times 1:2 in Reagent Buffer Plus Inhibitors over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a line with a slope of 1.014 and correlation coefficient of 0.998.

Precision

Intra-assay precision was determined by assaying 20 replicates of 3 buffer controls containing pERK in a single assay. Inter-assay precision was determined by measuring buffer controls of varying pERK concentrations in multiple assays over several days.

Intra-assay	pERK (pg/mL)	%CV
Low	190.9	3.7
Medium	384.3	2.9
High	1099.7	4.4

Inter-assay	pERK (pg/mL)	%CV
Low	187.0	7.8
Medium	367.2	5.6
High	1121.6	5.5

Cross-reactivity

The ERK1/2 [pThr^{202}/Tyr^{204}] ELISA Kit is specific for bioactive pERK.

Compound	Cross Reactivity
• Non-phosphorylated ERK • Phospho p38 • Non-phosphorylated p38 • Phospho-JNK • Non-phosphorylated JNK • Phospho-AKT • Non-phosphorylated AKT	<0.01%

Sample recovery

pERK concentrations were measured in Cell Lysis Buffer. pERK was spiked into the undiluted samples of these matrices which were then diluted with the reagent diluent concentrate and assayed in the kit.

Sample	% Recovery	Recommended Dilution
Cell Lysis Buffer	103.6%	1:8

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

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