> ERK1/2 EIA kit

Catalog # ADI-900-152

96 Well Enzyme Immunoassay Kit
For use with cell lysates

All reagents, except standard, should be stored at 4˚C. Store standard at -20˚C.

Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

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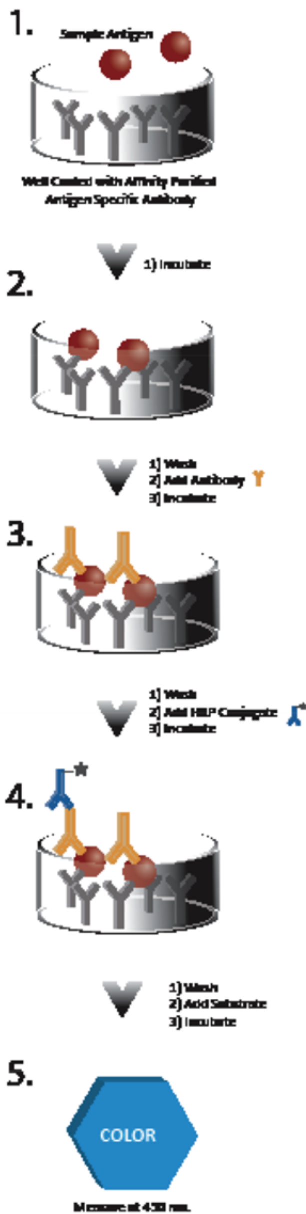

Introduction

The Erk1/2 EIA kit is a complete kit for the quantitative determination of total ERK1/2 in cell lysates. Please read the complete kit insert before performing this assay.

ERK1 and ERK2 constitute a subset of the mitogen-activated protein kinase (MAPK) pathway. Also called p44 and p42, respectively, these molecules function to connect cell surface receptors to nuclear transcription factors, which in turn regulate and control a number of fundamental cellular processes. ERK1 and ERK2 may be stimulated by cytokines, growth factors, irradiation, osmolarity or temperature fluctuations, or physical stress. The upstream MAP kinases MEK1 and MEK2 activate ERK1 and ERK2 via dual phosphorylation at the conserved T-E-Y motif found at the human Thr202/Tyr204 sequence or the rat and mouse Thr183/Tyr185 sequence[1-2]. Activated MAP kinases translocate to the nucleus, where they may phosphorylate a wide range of transcription factors that regulate processes including proliferation, apoptosis, survival, and differentiation. The literature contains numerous and extensive reviews on MAP kinases[3-9].

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody specific for ERK1/2. The plate is then incubated.

2. The plate is washed, leaving only bound ERK1/2 on the plate. A yellow solution of polyclonal antibody to Total ERK1/2 is then added. This binds the ERK1/2 captured on the plate. The plate is then incubated.

3. The plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the Total ERK1/2 polyclonal. The plate is again incubated.

4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. The substrate generates a blue color when catalyzed by the HRP.

5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of ERK1/2 in the sample.

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Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known effects of the molecule.

Materials Supplied

1. Assay Buffer 21
   100 mL, Catalog No. 80-1519
   Tris buffered saline containing detergents.

2. ERK Standard
   0.25 mL, Catalog No. 80-0993
   One vial containing 200,000 pg/mL of recombinant human ERK2.

3. RIPA Cell Lysis Buffer 2
   100 mL, Catalog No. 80-1284
   50 mM Tris HCl, pH7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS.

4. ERK Clear Microtiter Plate
   One Plate of 96 Wells, Catalog No. 80-0936
   A plate of break-apart strips coated with a mouse monoclonal antibody raised against a synthetic peptide derived from sequence near the carboxy-terminus of rat ERK1.

5. Wash Buffer Concentrate
   100 mL, Catalog No. 80-1287
   Tris buffered saline containing detergents.

6. Total ERK1/2 EIA Antibody
   10 mL, Catalog No. 80-1459
   A yellow solution of rabbit polyclonal antibody to ERK1/2 raised against a synthetic peptide derived from sequence near the carboxy-terminus of rat ERK 1/2.

7. Total ERK1/2 EIA Conjugate
   10 mL, Catalog No. 80-1460
   A blue solution of anti-rabbit IgG conjugated to horseradish peroxidase.

Activity of conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.

8. TMB Substrate
   10 mL, Catalog No. 80-0350
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.

Protect substrate from prolonged exposure to light.

9. Stop Solution 2
   10 mL, Catalog No. 80-0377
   A 1N solution of hydrochloric acid in water.

Stop solution is caustic. Keep tightly capped.

10. Total ERK Assay Layout Sheet
    1 each, Catalog No. 30-0232

11. Plate Sealer
    3 each, Catalog No. 30-0012

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Reagents require separate storage conditions.

Storage

All components of this kit, except the standard, are stable at 4ºC until the kit's expiration date. The standard must be stored at or below -20ºC.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Phenylmethylsulfonyl fluoride (PMSF), Sigma #P7626 or equivalent.
3. Protease inhibitor cocktail (PIC), Sigma #P8340 or equivalent.
4. Precision pipets for volumes between 5 µL and 1,000 µL.
5. Repeater pipet for dispensing 100 µL.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Lint-free paper for blotting.
10. Microplate reader capable of reading at 450 nm.
11. Graph paper for plotting the standard curve.

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Reagent Preparation

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

1. **Wash Buffer**

   Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit's expiration, or for 3 months, whichever is earlier.

If inhibitors other than those recommended are used, the end-user is responsible for assay validation. In some cases, certain protease inhibitor cocktails may cause performance differences.

2. **PIC and PMSF Addition.**

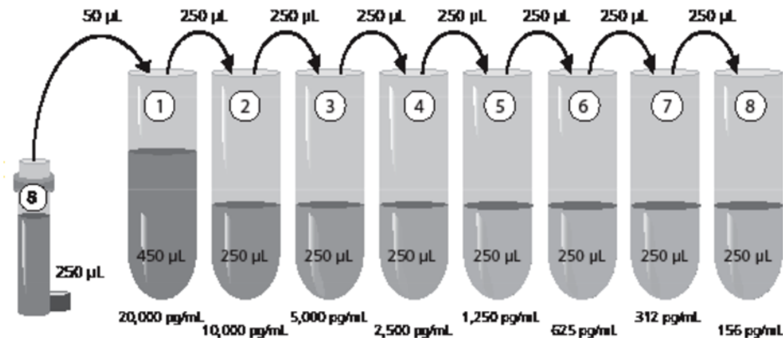
   Immediately prior to use, PIC and PMSF must be added to the assay buffer and RIPA Cell Lysis Buffer 2. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL PIC, or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.

   Inhibitors must be freshly added to the assay buffer and RIPA Cell Lysis Buffer 2 to ensure optimal integrity of the standards and samples. Each inhibitor treated buffer should incubate for 5-10 minutes at room temperature before it is used. Buffers treated with inhibitors should be used within 1 hour of preparation.

   Note: If the same cell lysates will be used in our phospho-ERK Enzyme Immunoassay Kit (900-098), phosphatase inhibitors must be added to the RIPA Cell Lysis Buffer 2 in addition to the recommended protease inhibitors (PIC and PMSF). Add sodium orthovanadate, such as Sigma #S6508, to a final concentration of 2 mM and sodium pyrophosphate, such as Sigma #S6422, to a final concentration of 20 mM.

Plastic tubes must be used for standard preparation.

3. **ERK Standards**



   Label eight 12 x 75 mm polypropylene tubes #1 through #8. Pipet 450 µL of the assay buffer into tube #1. Pipet 250 µL of the assay buffer into tubes #2 through #8. Add 50 µL of the 200,000 pg/mL standard stock into tube #1 and vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #8.

   Diluted standards should be used within 1 hour of preparation.

   The concentrations of ERK in tubes are labeled above.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Samples must be stored frozen at or below -20°C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Sample Handling

This assay is suitable for measuring total ERK in a wide range of cell lysates. Prior to assay, frozen samples should be slowly brought to 4ºC and centrifuged, if necessary, to isolate residual cell debris.

A minimum 1:4 dilution is required for RIPA Cell Lysis Buffer 2 to remove matrix interference of this buffer. Due to differences in cell types, number of cells, or total cellular protein concentration, lysates may require greater dilution with assay buffer plus inhibitors to remove interference or to be read within the standard range. Below are examples of the lysis of human Jurkat cells, mouse 3T3 cells, and rat C6 cells:

Sample	# cells per mL of lysis buffer	Total cellular protein (mg/mL)	% Recovery	Recommended Dilution
Jurkat cells	2.6 million	0.35	92%	1:20
mouse 3T3 cells	5 million	3.39	95%	1:57
rat C6 cells	5 million	1.21	98%	1:61

Add PIC/PMSF to buffers prior to preparing samples.

Protocol for Cell Lysis

1. Harvest cells and centrifuge at 1,400 rpm for 7 minutes at 4ºC. Discard supernatant.
2. Resuspend pellet and wash with Hank's Balanced Salt Solution.
3. Centrifuge at 1,400 rpm for 7 minutes at 4ºC. Discard supernatant.
4. Resuspend pellet with RIPA Cell Lysis Buffer 2 plus inhibitors. Vortex and incubate on ice for 30 minutes.
5. Centrifuge at 16,000 x g for 20 minutes at 4ºC. The supernatants can be stored at or below -20ºC or used immediately in the assay.

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Assay Procedure

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

All standards, controls, and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100 µL of the assay buffer into the S0 (0 pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #8 to the bottoms of the appropriate wells.
3. Pipet 100 µL of the samples to the bottoms of the appropriate wells.
4. Seal the plate. Incubate for 1 hour on a plate shaker (~500 rpm) at room temperature.
5. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 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.
6. Pipet 100 µL of yellow antibody into each well except the blank.
7. Seal the plate. Incubate for 1 hour on a plate shaker (~500 rpm) at room temperature.
8. Wash as above (Step 5).
9. Add 100 µL of blue conjugate to each well except the blank.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
11. Wash as above (Step 5).
12. Pipet 100 µL of substrate solution into each well.
13. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
14. Pipet 100 µL of stop solution into each well.
15. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of ERK in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the average Net OD 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. Using linear graph paper, plot the average Net OD for each standard versus ERK concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

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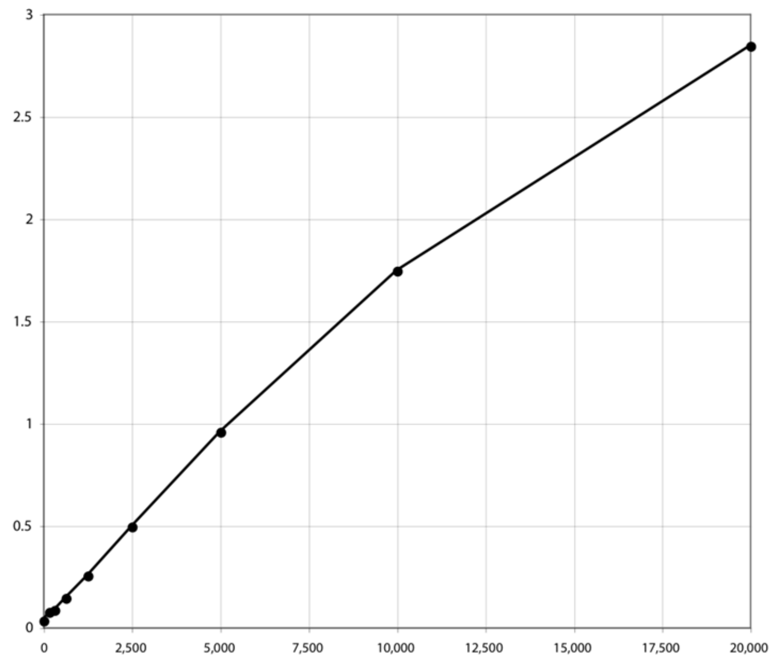
Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	ERK (pg/mL)
S0	0.018	**0**
S1	2.883	**20,000**
S2	1.750	**10,000**
S3	0.929	**5,000**
S4	0.456	**2,500**
S5	0.259	**1,250**
S6	0.129	**625**
S7	0.076	**312**
S8	0.045	**156**
Unknown 1	0.125	**632**
Unknown 2	1.458	**8105**



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Performance Characteristics

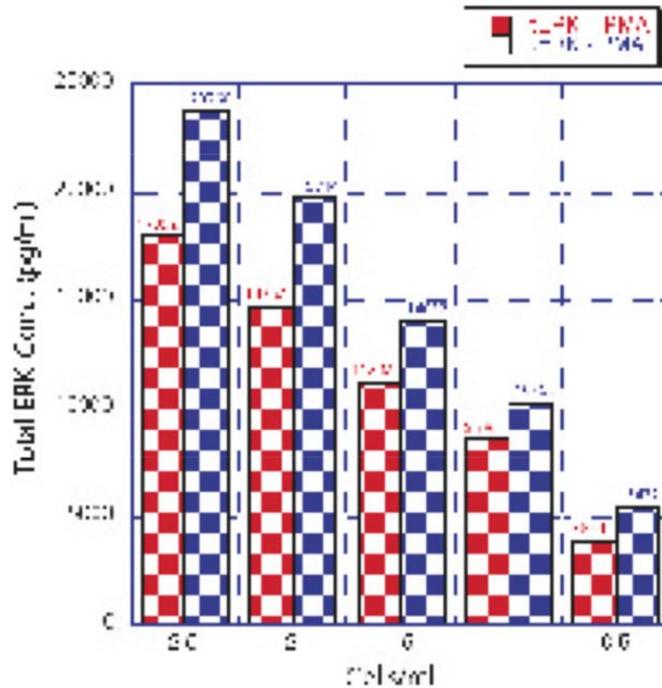
Specificity

The cross reactivities for a number of related compounds were determined by diluting cross reactants in the assay buffer at a concentration of either 20,000 pg/mL or 200,000 pg/mL. These samples were then measured in the assay.

Compound	Cross Reactivity
ERK1, active	100.0%
ERK2, active	100.0%
ERK1, inactive	100.0%
ERK2, inactive	92.6%
MEK1, inactive	<0.08%
MEK2, inactive	<0.08%
p38α, inactive	<0.08%
JNKα1α, inactive	<0.08%

Stimulation Experiment

2.5, 2.0, 1.5, 1.0, and 0.5 million Jurkat cells per mL were used in this experiment adapted from a protocol outlined in reference 10. Each cell concentration was treated for 5 minutes at 37ºC with either 50 nM PMA (a protein kinase activator) or DMSO (as an unstimulated control). The stimulated (+) and unstimulated (-) cells were lysed according to the protocol described on page 6. Lysates were run in the EIA and in the Western blots to generate the data illustrated. In the Western blot, 25 µL of cell lysate were loaded in each lane. 2.5 ng of Recombinant ERK2 were run as a positive control. The exposure time for development was 15 seconds for both the Total ERK 1/2 and the phospho ERK1/2 detection antibody.



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Sensitivity

Sensitivity was calculated as the ratio of the mean OD plus 2 standard deviations of twenty-four replicates of the 0 pg/mL standard to the mean of twenty-four replicates of the lowest standard, multiplied by the concentration of that standard (156 pg/mL). This value was determined to be 22.9 pg/mL.

Linearity

A buffer sample containing ERK was serially diluted 1:2 in kit assay buffer and measured in the assay. The results are shown in the table below.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	---	18,719 pg/mL	---
1:2	9,359 pg/mL	9,223 pg/mL	98.5 %
1:4	4,680 pg/mL	4,728 pg/mL	101.0 %
1:8	2,340 pg/mL	2,389 pg/mL	102.1 %
1:16	1,170 pg/mL	1,287 pg/mL	110.0 %
1:32	585 pg/mL	656 pg/mL	112.1 %
1:64	292 pg/mL	327 pg/mL	112.0 %
1:128	146 pg/mL	185 pg/mL	126.7 %

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing ERK in a single assay.

pg/mL	%CV
629	4.5
2,701	1.7
7,459	2.8

Inter-assay precision was determined by measuring buffer controls of varying ERK concentrations in multiple assays over several days.

pg/mL	%CV
646	10.4
2,789	7.3
8,417	5.8

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Notes

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Notes

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Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

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