# *Recombinant Human Active ERK2*

## *Certificate of Analysis*

**Catalog Number:** 1230-KS
**Lot Numbers:** 1500989

## *Specifications and Use*

| | |
|---|---|
| **Source** | ♦ Recombinant human ERK2 was expressed by *E. coli* using an N-terminal GST tag. The gene accession number is NM_002745. |
| **Molecular Mass** | ♦ The approximate molecular weight is 68 kDa (see Figure 1 below). |
| **Purity** | ♦ The purity was determined to be > 95% by densitometry (see Figure 1 below). |
| **Formulation** | ♦ Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 10 mM glutathione, 0.1 mM EDTA, 0.1 mM PMSF, and 25% Glycerol. |
| **Size** | ♦ 10 μg. |
| **Concentration** | ♦ 0.1 μg/μL. |
| **Activity** | ♦ The specific activity of ERK2 was determined to be 620 nmol/min/mg using a myelin basic protein (MBP) substrate (see Activity Assay Protocol). |
| **Storage** | ♦ This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature.<br>♦ **Avoid repeated freeze-thaw cycles.** |

## *ERK2*

ERK2 is a protein serine/threonine kinase that is a member of the extracellular signal-regulated kinases (ERKs) which are activated in response to numerous growth factors and cytokines (1). Activation of ERK2 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK2 is ubiquitously distributed in tissues with the highest expression in the heart, brain, and spinal cord. Activated ERK2 translocates into the nucleus where it phosphorylates various transcription factors (e.g., Elk-1, c-Myc, c-Jun, c-Fos, and C/EBPβ).

### *References*


1. Boulton, T.G. *et al*. (1991) Biochemistry **30**(1):278.


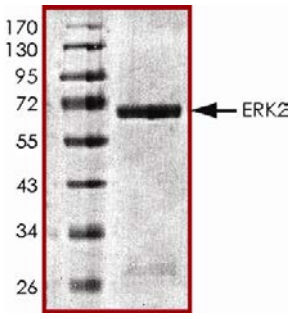


**Figure 1:** The approximate molecular weight is 68 kDa and the purity is > 95%.

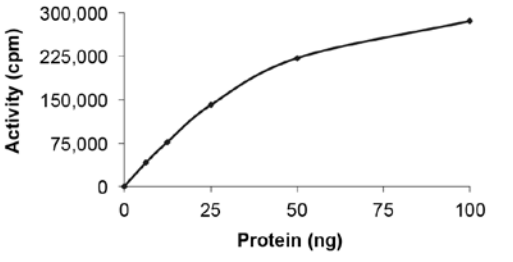


**Figure 2:** The specific activity of this lot of ERK2 was determined to be 620 nmol/min/mg as per the Activity Assay Protocol (on reverse).




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## *Activity Assay Protocol*

**Solutions Required**

- **Active Kinase** - Active ERK2 (0.1 μg/μL) diluted with Kinase Dilution Buffer III. **Note:** *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer III** - Kinase Assay Buffer I diluted 5-fold with 50 ng/μL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 μL aliquots at ≤ -20 °C.
- **[$^{33}$P]-ATP Assay Cocktail** - Prepare 250 μM [$^{33}$P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 μL of 10 mM ATP Stock Solution, 100 μL of [$^{33}$P]-ATP (1 mCi/100 μL), and 5.75 mL of Kinase Assay Buffer I. Store 1.0 mL aliquots at ≤ -20 °C.
- **Substrate** - Myelin Basic Protein (MBP) substrate diluted in distilled or deionized water to a final concentration of 1.0 mg/mL.

**Assay Procedure**

1. Thaw the [$^{33}$P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active ERK2, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer III on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μL.

| Reaction Component | Amount |
|---|---|
| Diluted Active ERK2 | 10 μL |
| MBP Substrate (1 mg/mL; on ice) | 5.0 μL |
| Distilled or deionized water (on ice) | 5.0 μL |

4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled or deionized water.
5. Initiate the reaction with the addition of 5.0 μL [$^{33}$P]-ATP Assay Cocktail, bringing the final volume up to 25 μL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15-minute incubation, terminate the reaction by spotting 20 μL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

**Calculation of [$^{33}$P]-ATP Specific Activity (SA) (cpm/pmol)**
Specific Activity (SA) = cpm for 5.0 μL [$^{33}$P]-ATP/pmole of ATP (in 5.0 μL of a 250 μM ATP stock solution, *i.e*. 1250 pmoles)

**Calculation of Kinase Specific Activity (SA) (pmol/minutes/μg or nmol/minutes/mg)**
Corrected cpm from reaction / [(SA of $^{33}$P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in μg or mg)] x [(Reaction volume) / (Spot Volume)]

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