

## Certificate of Analysis

### PKA, catalytic subunit, recombinant (Recombinant enzyme expressed in *E.coli* cells)

Item # 14-440, 14-440-K, 14-440M

Parent Lot # D8MN079U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** Untagged, recombinant full-length human PKA, catalytic subunit type alpha, expressed in *E.coli* cells. Purified using phosphocellulose P11 followed by gel filtration. Purity 97.8% by SDS-PAGE and Coomassie blue staining. MW = 41kDa.

**Specific Activity (Parent lot# D8MN079U):** 8580U/mg, where one unit of PKA activity is defined as 1nmol phosphate incorporated into 30µM Kemptide per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 0.432mg/ml of enzyme in 30mM potassium phosphate pH7.4, 150mM KCl, 1mM EDTA, 1mM DTT, 50% glycerol. Frozen solution.

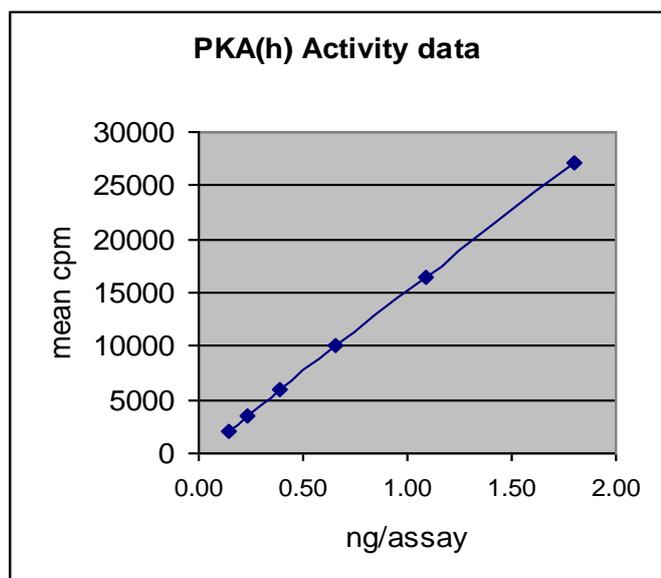
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

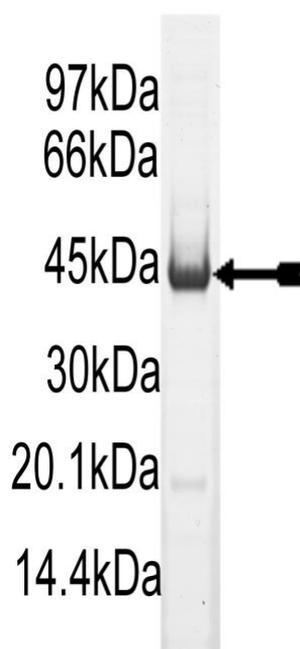
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 0.1–1.8ng of this lot of enzyme phosphorylated 30µM Kemptide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



**MS Tryptic Fingerprint:** Confirmed identity as PKA with the translated sequence listed on page three.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PKA.

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### Kinase Assay Protocol

#### Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS, pH7.0, 1mM EDTA.
- 2. Kemptide:** Use at a final concentration of 30 $\mu$ M. Dilute with reaction buffer for a 300 $\mu$ M stock. Use 2.5 $\mu$ l of stock per assay point.
- 3. PKA, active:** Dilute with 20mM MOPS, pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.1–1.8ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **kemptide**.
3. Add **2.5 $\mu$ l (0.1–1.8ng) PKA, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid
8. Transfer a 10 $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 $\mu$ l of 30% phosphoric acid.

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### PKA Sequence Information

<b><u>Protein</u></b>	Human PKA, catalytic subunit type alpha
<b><u>Tags</u></b>	untagged
<b><u>Native sequence</u></b>	M1 of the recombinant protein is equivalent to M1 of human PKA
<b><u>Accession number</u></b>	GenBank X07767

#### Recombinant PKA amino acid sequence:

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1  MGNAAAAKKG  SEQESVKEFL  AKAKEDFLKK  WESPAQNTAH  LDQFERIKTL  GTGSFGRVML
61  VKHKETGNHY  AMKILDKQKV  VKLKQIEHTL  NEKRILQAVN  FPFLVKLEFS  FKDNSNLYMV
121 MEYVPGGEMF  SHLRRIGRFS  EPHARFYAAQ  IVLTFEYLHS  LDLIYRDLKP  ENLLIDQQGY
181 IQVTDGFAK  RVKGRWTWTL  GTPEYLAPEI  ILSKGYNKAV  DWWALGVLIY  EMAAGYPPFF
241 ADQPIQIYEK  IVSGKVRFPS  HFSSDLKDLL  RNLLQVDLTK  RFGNLKNGVN  DIKNHKWFAT
301 TDWIAIYQRK  VEAPFIPKFK  GPGDTSNFDD  YEEEEIRVSI  NEKCGKEFSE  F

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#### Recombinant PKA nucleotide sequence:

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1  atgggcaacg  cgcgccggcg  caagaagggc  agcgagcagg  agagcgtgaa  agaattctta
61  gccaaagcca  aagaagattt  tcttaaaaaa  tgggaaagtc  ccgctcagaa  cacagcccac
121 ttggatcagt  ttgaacgaat  caagaccctc  ggcacgggct  ccttcgggcg  ggtgatqctg
181 gtgaaacaca  aggagaccgg  gaaccactat  gccatgaaga  tcctcgacaa  acagaaggtg
241 gtgaaactga  aacagatcga  acacaccctg  aatgaaaagc  gcatacctgca  agctgtcaac
301 tttccgttcc  tcgtcaaact  cgagttctcc  ttcaaggaca  actcaaactt  atacatggtc
361 atggagtacg  tgcccggcgg  ggagatgttc  tcacacctac  ggcggatcgg  aaggttcagt
421 gagccccatg  cccgtttcta  cgcgggccag  atcgtcctga  cttttgagta  tctgcaactg
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901 actgactgga  ttgccatcta  ccagaggaag  gtggaagctc  ctttcatacc  aaagtttaaa
961 ggccctgggg  atacgagtaa  ctttgacgac  tatgaggaag  aagaaatccg  ggtctccatc
1021 aatgagaagt  gtggcaagga  gttttctgag  ttttag

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Reviewed and approved by site quality representative.

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