abcam

# Kinase assay protocol

For ab125560

Version 1 Last updated 29 April 2020

# Kinase assay protocol for PDHK4 Abcam ab125560

1. Thaw [33P]-ATP assay cocktail in shielded container in a designated radioactive working area.

2. Thaw the Active PDHK4, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

3. In a pre-cooled microfuge tube, add the following reaction components, bringing the initial reaction volume up to 20µl.

Component 1. 10µl of diluted active PDHK4 (ab125560)
Component 2. 10µl of 0.2µg/ul stock solution of substrate (ab125602)

4. Set up blank control as outlined in step 3, excluding the addition of substrate. Replace the substrate with an equal volume of distilled water.

5. Initiate the reaction by the addition of 5 µl [33P]-ATP Assay Cocktail bringing the final volume up to 25 µl and incubate the mixture in a water bath at 30oC for 15 minutes.

6. After the 15 minute incubation period, 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 (dilute 10 ml of phosphoric acid and make a 1 L solution with distilled H2O) with constant gentle stirring. It is recommended that the strips should be washed a total of 3 intervals 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 removing the blank control value (see step 4) for each sample and calculate the kinase specific activity as below:

**Calculation of [P33]-ATP Specific Activity (SA) (cpm/pmol)**

Specific activity (SA) = cpm for 5 µl [33P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

**Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)**

Corrected cpm from reaction / (Specific activity of [33P]-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg) x [(Reaction volume / spot volume)]

### Active Kinase

Active PDHK4 ab125560, 0.1µg/µl diluted with Kinase Dilution Buffer III (ab189134), assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active PDHK4 for optimal results).

### Kinase Dilution Buffer (ab189134)

Kinase Assay Buffer I (ab189135) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

### Kinase Assay Buffer I (ab189135)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [$^{33}$P]-ATP Assay Cocktail

Prepare 250µM [$^{33}$P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (ab189136), 100 µl [$^{33P}$]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (ab189135). Store 1ml aliquots at –20°C.

### 10mM ATP Stock Solution (ab189136)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (ab189135). Store 200µl aliquots at –20°C.

### Substrate (ab125602)

PDHA1 substrate ab125602 prepared in buffer (50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.25mM DTT, 25% glycerol) to a final concentration of 0.2µg/µl.