SIGMA-ALDRICH®

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Product Information

ULK3, active, His tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5098**
Storage Temperature –70 °C

Synonym: DKFZP434C131

Product Description

ULK3 or unc-51-like kinase 3 is a member of the serine/threonine kinase family that is involved in the SHH signaling pathway as a positive regulator of GLI proteins.[1] ULK3 enhances endogenous and over-expressed GLI transcriptional activity in cultured cells and alters its subcellular localization. Furthermore, ULK3 phosphorylates GLI proteins *in vitro*. ULK3 is widely expressed and its expression is higher in a number of tissues where SHH signaling is known to be active, again suggesting ULK3 is involved in the SHH pathway as a positive regulator of GLI proteins.

Recombinant, full-length, human ULK3 was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His tag. The gene accession number is BC157884. Recombinant protein stored in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.25 mM DTT, and 25% glycerol.

Molecular mass: ~51 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 195–265 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

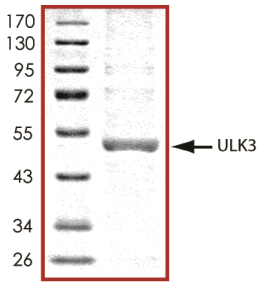
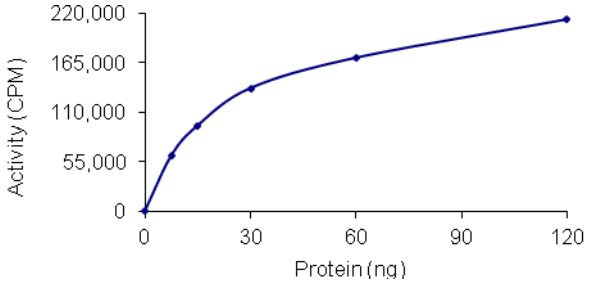


Figure 2.
Specific Activity of Typical Lot
195–265 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA.

Kinase Solution – Dilute the active ULK3 (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration. Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active ULK3 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at –20 °C.

γ-^{33}P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μl of 10 mM ATP Stock Solution, 100 μl of γ-^{33}P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the protein substrate in distilled water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ^{33}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active ULK3, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{33}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μl:
   - 10 μl of Kinase Solution
   - 5 μl of Substrate Solution
   - 5 μl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 μl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μl of the γ-^{33}P-ATP Assay Cocktail, bringing the final reaction volume to 25 μl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{33}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{33}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
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 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μl of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Maloverjan, A. et al., Identification of a novel serine/threonine kinase ULK3 as a positive regulator of Hedgehog pathway. Exp. Cell Res., **316**, 627-637 (2010).

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