Product Information

LATS2, active, GST tagged, human
PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number SRP5212
Storage Temperature –70 °C

Product Description
LATS2 is a serine/threonine protein kinase belonging to the LATS tumor suppressor family. LATS2 interacts with a negative regulator of p53 and functions in a positive feedback loop with p53 that responds to cytoskeleton damage and this interaction provokes centrosome/mitotic apparatus dysfunction. LATS2 plays an essential role in the maintenance of mitotic fidelity and genomic integrity.

Recombinant human LATS2 (480-1088) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The LATS2 gene accession number is NM_014572. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~110 kDa
Purity: 70–95% (SDS-PAGE, see Figure 1)
Specific Activity: 9.4–12.6 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
9.4–12.6 nmole/min/mg

Procedure
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl2, 5 mM EGTA, 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 LATS2 (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 LATS2 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.

γ³³P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ³³P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at −20 °C.

Substrate Solution – Dissolve the synthetic peptide 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 ³³P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active LATS2, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ³³P-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 γ³³P-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 γ³³P-ATP counts introduced into the reaction. Spot 5 µl of the γ³³P-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)

\[
SR = \frac{\text{cpm of } 5 \mu l \text{ of } \gamma^{33}\text{P}-\text{ATP Assay Cocktail}}{\text{nmole of ATP}}
\]

\[
cpm \text{ – value from control (step 7)}
\]

\[
nmole \text{ – 1.25 nmole (5 } \mu l \text{ of 250 } \mu M \text{ ATP Assay Cocktail)}
\]

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

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

\[
SR = \text{specific radioactivity of the ATP (cpm/nmole ATP)}
\]

\[
\Delta \text{cpm} = \text{cpm of the sample – cpm of the blank (step 3)}
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\[
25 = \text{total reaction volume}
\]

\[
20 = \text{spot volume}
\]

\[
T = \text{reaction time (minutes)}
\]

\[
E = \text{amount of enzyme (mg)}
\]

**References**


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