

## Product Information

**TGFβR2, Active**  
**human, recombinant**  
**GST-tagged, expressed in Sf9 cells**

Catalog Number **T0203**  
Lot Number 019K1631  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

Synonyms: AAT3; FAA3; MFS2; RIIC; HNPCC6;  
TGFR-2; TGFbeta-RII; TAAD2

### Product Description

TGFβR2 is a member of the TGFβ receptor subfamily and is a Ser/Thr protein kinase. TGFβR2-induced protein phosphorylation plays a key role in signal transduction that leads to mitogenic responses.<sup>1</sup> The TGFβR2 receptor transmits signals from the cell surface to the nucleus and provides instructions for making transforming growth factor (TGF)-β type II receptor. Mutations in TGFβR2 gene have been associated with Marfan Syndrome, Loeys-Deitz Aortic Aneurysm Syndrome, and the development of various types of tumors.<sup>2</sup>

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 003242. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~68 kDa

Purity:  $\geq 70\%$  (SDS-PAGE, see Figure 1)

Specific Activity: 6–8 nmole/min/mg (see Figure 2)

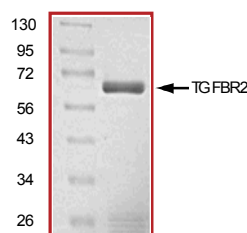
### Precautions and Disclaimer

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.

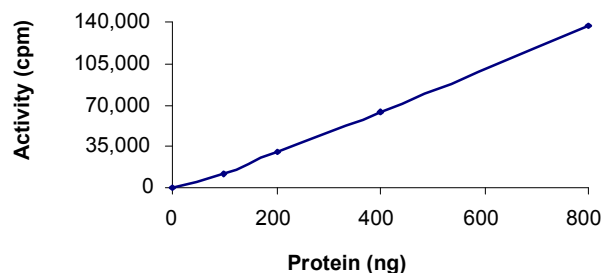
### Storage/Stability

The product ships on dry ice and storage at  $-70\text{ }^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70\text{ }^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Lot Number 019K1631:  
>90% (densitometry)



**Figure 2.**  
Specific Activity of Lot Number 019K1631:  
7 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl<sub>2</sub>, 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 solution.

Kinase Solution – Dilute the Active TGFβR2 (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active TGFβR2 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-<sup>32</sup>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-<sup>32</sup>P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the myelin basic protein (MBP) in 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 <sup>32</sup>P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active TGFβR2, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-<sup>32</sup>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-<sup>32</sup>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 pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut 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-<sup>32</sup>P-ATP counts introduced into the reaction. Spot 5 μl of the γ-<sup>32</sup>P-ATP Assay Cocktail on a pre-cut 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)

$$SR = \frac{\text{cpm of } 5 \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

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. Cheng, N. et al., Enhanced hepatocyte growth factor signaling by type II transforming growth factor-beta receptor knockout fibroblasts promotes mammary tumorigenesis. *Cancer Res.*, **67**, 4869-4877 (2007).
2. Sakai, H. et al., Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan-related phenotypes. *Am. J. Med. Genet.*, **140**, 1719-1725 (2006).

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