

# Product Information

## **IRAK2, active, GST tagged, human, PRECISIO® Kinase, recombinant, expressed in baculovirus infected Sf9 cells**

Catalog Number **I2660**

Lot Number 061M0760

Storage Temperature –70 °C

Synonyms: IRAK-2, MGC150550

### **Product Description**

Interleukin-1 receptor-associated kinase 2 (IRAK2) is an important downstream signaling component of Toll-like receptors (TLRs).<sup>1</sup> IRAKs were first described as signal transducers for IL-1 and later have been implicated in signal transduction of other members of the Toll/IL-1 receptor family. The interleukin-1 receptor (IL-1R) signaling pathway leads to NF $\kappa$ B activation in mammals. To date, four mammalian IRAKs have been identified (IRAK-1, IRAK-2, IRAK-4, and IRAK-M).<sup>2</sup> It is believed that IRAK-2 could be an additional therapeutic target for inhibiting IL-1 induced inflammation.

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM 001570. 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: ~103 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 5–7 nmole/min/mg (see Figure 2)

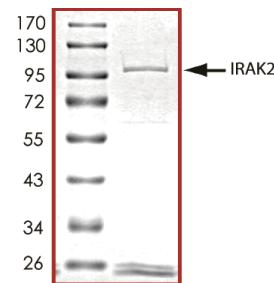
### **Precautions and Disclaimer**

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.

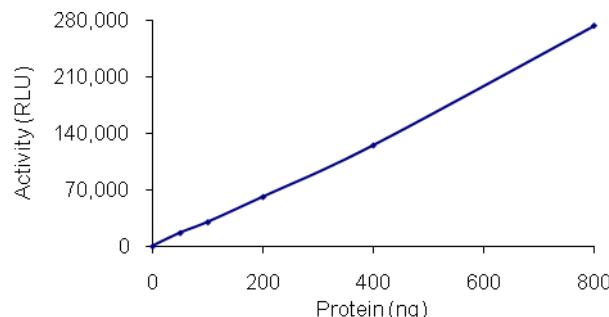
### **Storage/Stability**

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 Lot Number 061M0760:  
>75% (densitometry)



**Figure 2.**  
Specific Activity of Lot Number 061M0760:  
6 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 water.

Kinase Solution – Dilute the active IRAK2 (0.1  $\mu$ g/ $\mu$ L) 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 the researcher perform a serial dilution of active IRAK2 kinase for optimal results.

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

$\gamma$ -<sup>32</sup>P-ATP Assay Cocktail (250  $\mu$ M) – Combine 5.75 ml of Kinase Assay Buffer, 150  $\mu$ L of 10 mM ATP Stock Solution, 100  $\mu$ L of  $\gamma$ -<sup>32</sup>P-ATP (1 mCi/100  $\mu$ L). 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 IRAK2, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The  $\gamma$ -<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  $\mu$ L:  
10  $\mu$ L of Kinase Solution  
5  $\mu$ L of Substrate Solution  
5  $\mu$ L of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5  $\mu$ L of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5  $\mu$ L of the  $\gamma$ -<sup>32</sup>P-ATP Assay Cocktail, bringing the final reaction volume to 25  $\mu$ L. 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  $\mu$ L 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  $\gamma$ -<sup>32</sup>P-ATP counts introduced into the reaction. Spot 5  $\mu$ L of the  $\gamma$ -<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  $\mu$ L of 250  $\mu$ M 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)

$\Delta$ cpm = 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. Muzio, M. et al., IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science*, **278**, 1612-1615 (1997).
2. Wesche, H. et al., IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. *J. Biol. Chem.*, **274**, 19403-19410 (1999).

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