

## HER4, Active

Recombinant human protein expressed in Sf9 cells

Catalog # E29-11G-10

Lot # L290-1

### Product Description

Recombinant human HER4 (682-993) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_005235](#).

### Gene Aliases

ERBB4, MGC138404, p180erbB4

### Concentration

0.1 µg/µl

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage, Shipping and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

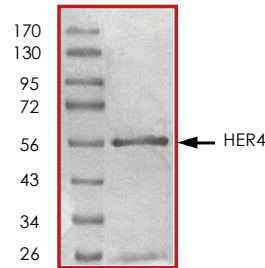
### Scientific Background

HER4 or ERBB4 is a transmembrane receptor tyrosine kinase that belongs to epidermal growth factor receptor family (1). Through its interaction with its activating ligand heregulin, HER4 can regulate proliferation and differentiation of cells (2). HER4 ectodomain is cleaved by a metalloprotease, gamma-secretase that releases the HER4 intracellular domain from the membrane and facilitates its translocation to the nucleus. The kinase activity of HER4 is both necessary and sufficient to trigger an antiproliferative response in human breast cancer cells. Increased expression of HER4 has been detected in various cancers and this usually correlates to better survival.

### References

1. Carolyn, I. et al: HER4 Mediates Ligand-Dependent Antiproliferative and Differentiation Responses in Human Breast Cancer Cells. *Mol.Cellular Biol.*, 2001, 21: 4265-4275.
2. Jorma, A. et al: Proteolytic Cleavage and Phosphorylation of a Tumor-associated ErbB4 Isoform Promote Ligand-independent Survival and Cancer Cell Growth. *MolBiolCell*, 2006; 17, 1: 67-79.

### Purity

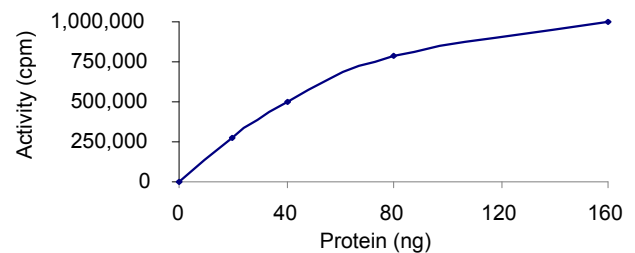


**Figure 1. SDS-PAGE gel image**

The purity of HER4 was determined to be **>90%** by densitometry, approx. MW **57kDa**.

### Specific Activity

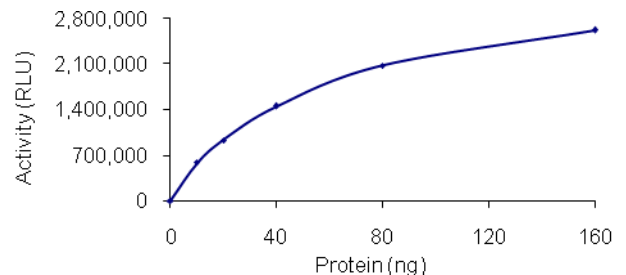
**Figure 2. Radiometric Assay Data**



The specific activity of HER4 was determined to be **669 nmol /min/mg** as per activity assay protocol.

(For Radiometric Assay Protocol on this product please see pg. 2)

**Figure 3. ADP- Glo™ Assay Data**



The specific activity of HER4 was determined to be **770 nmol /min/mg** as per activity assay protocol.

(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: E29-11G-10)

Active HER4 (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) and 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 HER4 for optimal results).

### Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/µl BSA solution.

### Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 20mM MgCl<sub>2</sub>, 12.5mM MnCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: P61-58)

Poly (Glu<sub>4</sub>,Tyr<sub>1</sub>) synthetic peptide substrate diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active HER4, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 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 HER4 (Catalog #E29-11G-10)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #P61-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity (cpm) on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

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

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

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

# ADP-Glo™ Activity Assay Protocol

## Reaction Components

### HER4 Kinase Enzyme System (Promega, Catalog #:V3101)

HER4, Active, 10µg (0.1µg/µl)  
Poly (Glu<sub>4</sub>,Tyr<sub>1</sub>) peptide, substrate, 1ml (1mg/ml)  
Reaction Buffer A (5X), 1.5ml  
DTT solution (0.1M), 25µl  
MnCl<sub>2</sub> solution (2.5M), 25µl

### ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10mM (0.5ml)  
ADP, 10mM (0.5ml)  
ADP-Glo™ Reagent (5ml)  
Kinase Detection Buffer (10ml)  
Kinase Detection Substrate (Lyophilized)

### Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl<sub>2</sub> and 0.5 mg/ml BSA.

## Assay Protocol

The HER4 assay is performed using the HER4 Kinase Enzyme System (Promega; Catalog #: V3101) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The HER4 reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html).

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of HER4 Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT, 1.6µl MnCl<sub>2</sub> and 597.4µl of dH<sub>2</sub>O.
- Step 4.** Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH<sub>2</sub>O.
- Step 5.** Prepare diluted HER4 in 1X Buffer (diluted from 2X buffer) 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 HER4 for optimal results).
- Step 6.** In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

<b>Component 1.</b>	10µl of diluted Active HER4
<b>Component 2.</b>	5µl of 1mg/ml stock solution of substrate
<b>Component 3.</b>	5µl of 2X Buffer
- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 8.** At the same time as the HER4 kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the ADP-Glo™ Kinase Assay technical Manual #TM313.
- Step 9.** Initiate the HER4 reactions by the addition of 5µl of 250µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at <http://www.promega.com/applications/cellularanalysis/cellsignaling.htm>

### Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol / (Reaction time in min)\*(Enzyme amount in mg)