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# ProductInformation

**INSULIN RECEPTOR FROM RAT LIVER**
Product Number **I 9266**
Storage Temperature –70 °C

## Product Description

Solution in 50% glycerol containing 50 mM HEPES, pH 7.6, 150 mM NaCl and 0.1% Triton, X-100.

The Insulin Receptor is a transmembrane protein which consists of 4 subunits (2α2β) and exhibits tyrosine kinase activity. Upon binding of insulin to the extracellular -subunit, the 95kD -subunit of tyrosine kinase is activated. The insulin receptor has been partially purified from rat liver membranes by affinity chromatography using lectin from *Triticum vulgaris* as the ligand.

Unit Definition: One unit of insulin activated insulin receptor will transfer 1.0 pmol of phosphate from [γ-$^{32}$P]ATP to Poly(Glu,Tyr) 4:1 per minute at 30 °C.

## Storage/Stability

Store the vial at –70 °C. At time of assay, thaw the vial contents on ice. Centrifuge at 1000 rpm for 2-3 minute at 2-8 °C to collect all of the solution at the bottom of the vial. Keep at 2-8 °C until the required samples are removed. The remainder of the solution can be stored at –70 °C after freezing it in liquid nitrogen. The activity of insulin receptor decreases after more than one thaw/freeze cycle.

## ASSAY REAGENTS AND PROCEDURE:

(Phosphorylation of Poly(Glu,Tyr) 4:1 by insulin receptor)

### Reagents:

1. [γ -$^{32}$P] ATP (10mCi/ml).

2. Enzyme Dilution Buffer:
   50 mM HEPES (Product No. H 3375), pH 7.6, containing 150 mM NaCl (Product No. S 5886) and 0.1% Triton X-100 (Product No. x100).

3. 2X Kinase Buffer:
   50 mM HEPES, pH 7.6, containing 50 mM MgCl2, 200 µM ATP (Product No. A 3377), 200 µM Sodium Orthovanadate (Product No. S 6508), 5 mg/ml Poly (Glu,Tyr) 4:1 (Product No. P 0275), and 50 µCi [γ-$^{32}$P]ATP/ml.

4. Insulin Dilution Buffer: 50 mM HEPES, pH 7.6, containing 100 µg/ml Bovine Serum Albumin (Product No. A 8022)

5. Insulin, Bovine (Product No. I 6634)
   Stock Solution: 0.1 mM in 0.01 M HCl.

   Working Solution: Dilute the Stock Solution 1:50 in Insulin Dilution Buffer to prepare the 2 µM Insulin Working Solution.

6. Whatman 3 mm chromatography paper cut into pieces of approx. 2 cm$^2$.

7. 10% Trichloroacetic Acid containing 10 mM Sodium Pyrophosphate, Decahydrate (Product No. S 9515).

8. Ethanol - Analytical Grade

9. Acetone - Analytical Grade

### Procedure:

Perform Steps 1-3 on ice.

1. Dilute the Insulin Receptor 5-fold in Enzyme Dilution Buffer (See note 1).

2. Mix 10 µl of diluted Insulin Receptor from Step 1 and 10 µl of Insulin Working Solution for enzyme activation. Mix 10 µl of diluted Insulin Receptor from Step 1 and 10 µl of Insulin Dilution Buffer for basal enzyme activity. Mix 10 µl of Enzyme Dilution Buffer and 10 µl of Insulin Dilution Buffer for blank.

3. Incubate for 30 minutes on ice.

4. Start the reaction by adding 20 µl of 2X Kinase Buffer.

5. Gently vortex for a few seconds and incubate for 10 minutes at 30 °C.

6. Apply a 30 µl aliquot of each reaction mixture from Step 5 to a piece of Whatman 3mm chromatography paper. Soak the pieces in 10% TCA containing 10mM Sodium Pyrophosphate at room temperature.

7. Wash the pieces of paper 4 times with 10% TCA containing 10mM Sodium Pyrophosphate: 10 ml of TCA solution are used per piece and per wash. Agitate gently throughout each wash for at least 15 minutes.

8. Wash once with ethanol (as in Step 7).

9. Wash once with acetone (as in Step 7).

10. Dry the paper pieces at room temperature or under a heat lamp and then count the radioactivity incorporated into precipitated Poly (Glu, Tyr) 4:1 using the Cerenkov mode (i.e. Count the β-emission without scintillation fluid using the $^{3}H$ channel).

**Calculations:**

Count R, the radioactivity of 10 µl of 2X Kinase Buffer, and multiply it by a factor of 2 in order to obtain the total radioactivity in cpm per assay tube: 2R

Divide the above value (2R) by the amount of ATP present in the assay tube (= 4000 pmoles), in order to obtain the specific radioactivity, SR:

$$SR = 2R/4000$$

Subtract the blank value from the counts of the sample and multiply the result by a factor of 4/3 in order to obtain the total counts per tube, C:

$$C = (Csample - Cblank) \times 4/3$$

**Activity:**

$C = (C_{sample} - C_{blank}) \times 4/3$

Units/ml = C/(SRx10 minX0.01 ml) X Dilution factor

The dilution factor is at least 5.

Notes:

1. The concentration of glycerol in the assay tube should not exceed 3%. Therefore, the minimal insulin receptor dilution possible is 5-fold (2.5% final glycerol concentration).
2. The activation of Insulin Receptor tyrosine kinase by insulin is approx. 5-fold.

Additional Information:

The autophosphorylation of the Insulin Receptor 95kD β-subunit is performed in a procedure similar to the above with some modifications:

1. The kinase buffer does not contain exogenous substrate [poly(Glu,Tyr) 4:1].
2. The specific radioactivity of the ATP is 3-4 fold higher.
3. The reaction is performed at room temperature.
4. The reaction is terminated by the addition of sample buffer.
5. The results are obtained by electrophoresis on 7.5% SDS/PAGE followed by autoradiography.

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