

Tyrosine Kinase Assay Kit

(non-radioactive)

Catalog # 17-315

Lot # 22167

Kit Components

Streptavidin Coated Strip Plate: Catalog #20-183, Lot # 22891. One streptavidin coated plate containing 12 strips of 8 wells each.

Poly (Glu₄-Tyr) Peptide, biotinylated: Catalog # 12-440A, Lot # 22095. See page two for more information. One vial containing **12.5µg** peptide in 250µl sterile distilled water.

Poly (Glu₄-Phospho-Tyr) Peptide, biotinylated: Catalog # 12-443, Lot # 22147. See page two for more information. One vial containing **5µg** in 50µl sterile 0.1M sodium bicarbonate.

Src Kinase Reaction Buffer (SrcRB), Catalog # 20-121. Lot #22777. Two vials, each containing **1ml** of a **4X** SrcRB: 100mM Tris-HCl, pH 7.2, 125mM MgCl₂, 25mM MnCl₂, 2mM EGTA, 250µM sodium orthovanadate, 2mM dithiothreitol.

Src Manganese/ATP Cocktail, Catalog # 20-110, Lot #23253. Two vials, each containing **1ml** of a **5X** Mn²⁺/ATP cocktail: 75mM manganese chloride and 500µM ATP in 20mM MOPS, pH 7.2, 25mM β-glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.

TBS/T, 20X, (ELISA wash buffer), Catalog # 20-202, Lot # 22096. One vial containing **50ml** of 1M Tris, 3M NaCl, 2% Tween-20, pH 7.4.

10% BSA in TBS, (ELISA blocking buffer), Catalog # 20-191, Lot # 22787. One vial containing **10ml** of 10% BSA in 50mM Tris, 150mM NaCl, pH 7.4.

Anti-phosphotyrosine, recombinant 4G10, HRP conjugate: Catalog # 16-184B, Lot # 22763. See page two for more information. One vial containing **25µl** of mouse IgG, conjugated to horseradish peroxidase, in PBS, 5mg/ml BSA, with 0.01% (w/v) merthiolate.

TMB (tetramethylbenzidine) Substrate Reagent A: Catalog # 20-182a, Lot #22788. One vial containing **7.5ml** of 0.04% (w/v) TMB solution.

TMB (tetramethylbenzidine) Substrate Reagent B: Catalog # 20-182b, Lot #22789. One vial containing **7.5ml** of 0.02% hydrogen peroxide in citric acid buffer.

**FOR RESEARCH USE ONLY. NOT RECOMMENDED OR INTENDED
FOR DIAGNOSIS OF DISEASE IN HUMANS.
DO NOT USE IN HUMANS.**

Quantity: Sufficient reagents for 96 assays per kit.

Storage: Upon receipt, store individual components at recommended temperatures. Store components 12-440A, 12-443, 20-121 and 20-110 at -20°C. Store all other components at 4°C.

Stability: Components stable for 6 months from date of shipment if stored and handled correctly.

Use: This non-radioactive kinase assay kit is a two-step peptide phosphorylation/colorimetric detection assay kit. In the first stage, a biotinylated substrate peptide containing tandem repeats of Poly (Glu₄-Tyr) is incubated with a tyrosine kinase enzyme sample in the presence of non-radioactive ATP and a Mn²⁺/Mg²⁺ co-factor cocktail. The kinase assay can be performed with substrate peptide either in solution, or bound to the well of a streptavidin-coated 96-well plate. The second step involves the detection of phosphorylated substrate by Direct Enzyme Linked Immunosorbent Assay (ELISA) using a monoclonal anti-phosphotyrosine-HRP (Horse Radish Peroxidase) antibody conjugate. Tetramethylbenzidine (TMB) is provided as the HRP substrate.

Please refer to the User Manual for further information and a detailed assay procedure.

Technical Information for Kit Components

Poly (Glu₄-Tyr) Peptide, biotinylated

Product Description: Fifty-four residue synthetic peptide [GG(EEEEEY)₁₀ EE] containing biotin at the N-terminus.

Molecular Weight = 7413 Daltons

Purity: Greater than 90% determined by reverse-phase HPLC.

Poly (Glu₄-Phospho-Tyr) Peptide, biotinylated

Product Description: Twenty-seven residue synthetic phospho-peptide [GG(EE[pY]EE)₅] containing a biotin at the N-terminus.

Molecular Weight = 4156 Daltons

Purity: Greater than 90% determined by reverse-phase HPLC.

**Anti-Phosphotyrosine, recombinant 4G10
HRP-Conjugate**

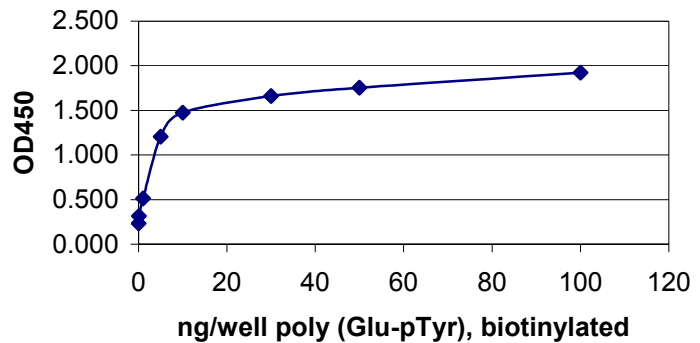
Product Description: Recombinant 4G10, produced in Chinese Hamster Ovary (CHO) cells expressing the 4G10 antibody heavy and light chain cDNAs. Heavy chain C-terminus has a hexa-histidine (His₆) tag for purification and immobilization via nickel affinity matrices. Cross-linked to horseradish peroxidase (HRP). Patent pending.

Immunogen: Phosphotyramine-KLH.

Quality Control Testing

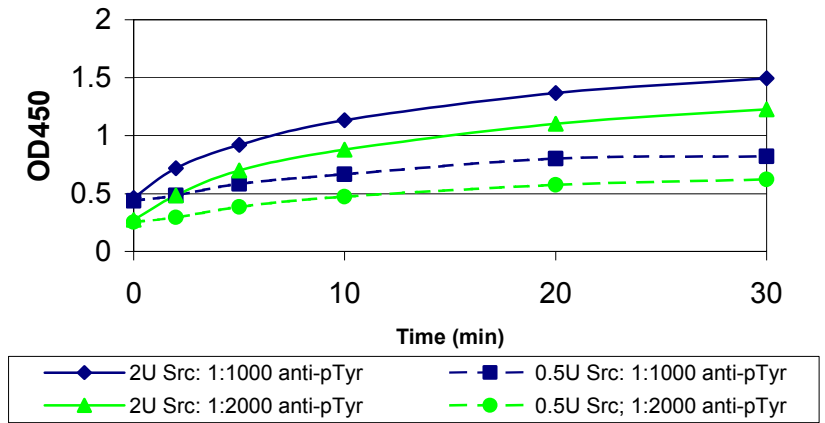
Reference Peptide Titration:

Increasing amounts of Poly (Glu₄-phospho-Tyr) Peptide, biotinylated were bound to streptavidin-coated wells. Total peptide mass/well was adjusted by the addition of Poly (Glu₄-Tyr) Peptide, biotinylated to a 100ng/well. Bound phospho-tyrosine containing peptide was detected with a 1:1000 dilution of anti-phospho-Tyrosine, recombinant 4G10, HRP conjugate.



Time Course with recombinant Src Enzyme:

0.5 and 2 Units of Src, active (Catalog # 14-326) were incubated for the indicated times in the presence of 100ng of Poly (Glu4-tyr) Peptide, biotinylated, Src Kinase Reaction buffer and Src Manganese/ATP Cocktail for the times indicated. Phosphotyrosine was detected in a direct ELISA assay using a 1:1000 or 1:2000 dilution of anti-phospho-Tyrosine, recombinant 4G10, HRP conjugate.



In vitro Kinase Assay:

10µg freshly prepared A431 modified RIPA cell lysate was incubated with the Poly (Glu₄-phospho-Tyr) Peptide, biotinylated, in the presence of Src Kinase Reaction buffer and Src Manganese/ATP Cocktail for the times indicated. Phosphotyrosine was detected in a direct ELISA assay using a 1:1000 dilution of anti-phosphotyrosine, recombinant 4G10, HRP conjugate.

