



cell signaling solutions

Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstate.com
Sales Department: T: 800 233-3991 • F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstate.com

Tyrosine Kinase Assay Kit, Colorimetric Detection

Catalog # 17-315

Lot # 27476

Kit Components

Streptavidin Coated Strip Plate, Catalog #20-183, Lot # 25292. One streptavidin coated plate containing 12 strips of 8 wells each.

Poly (Glu₄-Tyr) Peptide, biotin conjugate, Catalog # 12-440A, Lot # 27526A. See page two for more information. One vial containing **12.5µg** peptide in 250µl sterile distilled water.

Poly (Glu₄-Phospho-Tyr) Peptide, biotin conjugate, Catalog # 12-443, Lot # 27618. See page two for more information. One vial containing **5µg** in 50µl sterile 0.1M sodium bicarbonate.

Tyrosine Kinase Reaction Buffer, 5X, Catalog # 20-278. One vial containing **5ml** of 5X Tyrosine Kinase Reaction Buffer (TKRB): 100mM Tris-HCl, pH 7.4, 50mM MgCl₂, 5mM MnCl₂, 5mM dithiothreitol, 1mM ATP.

Sodium Orthovanadate, 50mM, Catalog # 20-279. One vial containing **500µl** of Sodium Orthovanadate, pH 10.0.

20X TBS, 2% Tween[®]-20, Catalog # 20-202. One vial containing **50ml** of 1M Tris, 3M NaCl, 2% Tween-20, pH 7.4.

10% BSA in TBS, Catalog # 20-191. 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 # 27480. See page two for more information. One vial containing **25µl** of IgG_{2bc}-HRP conjugate in PBS containing 5mg/ml BSA and 0.05% Kathon[®].

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

TMB (tetramethylbenzidine) Substrate Reagent B: Catalog # 20-182b. 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, 20-278 and 20-279 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, biotin conjugate

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, biotin conjugate

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**

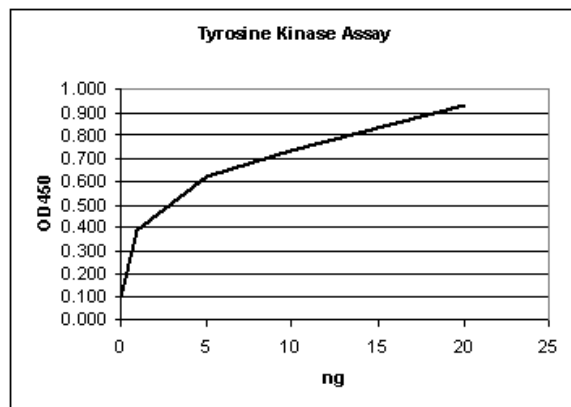
Product Description: Recombinant 4G10, produced in Chinese Hamster Ovary (CHO) cells expressing the 4G10 antibody heavy and light chain cDNAs. Heavy chain C-terminus has a hexa-histidine (His₆) tag for purification and immobilization via nickel affinity matrices. Cross-linked to horseradish peroxidase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

Immunogen: Phosphotyramine-KLH.

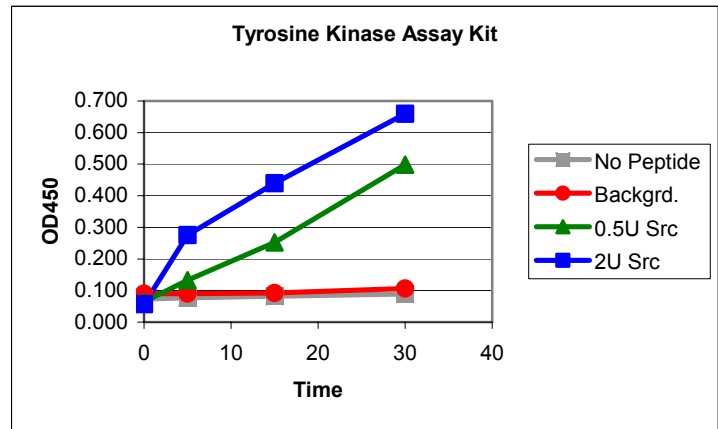
Quality Control Testing

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 1:2000 dilution of anti-phospho-Tyrosine, recombinant 4G10, HRP conjugate. Representative data from a previous lot is shown to the right.



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 1:2000 dilution of anti-phospho-Tyrosine, recombinant 4G10, HRP conjugate. Representative data from a previous lot is shown to the right.



In vitro Kinase Assay:

A previous lot was tested using 10µg freshly prepared A431 modified RIPA cell lysate was incubated with the Poly (Glu₄-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.

