Universal Tyrosine Kinase Assay Kit (Cat.# MK410)

Frequently Asked Questions: Universal Tyrosine Kinase Assay Kit

The Universal Tyrosine Kinase Assay Kit is an *in vitro* enzyme immunoassay kit for quantitative determination of tyrosine kinase activity using either adherent or suspension cell culture extracts, and is useful for analyzing regulation of PTK activity and for in vitro screening of PTK inhibitors. Applications for this kit include quantitative measurement of tyrosine kinase activity, analysis of PTK activity regulation, and to facilitate *in vitro* screening of known or potential PTK inhibitors.

Answers to frequently asked questions about the Universal Tyrosine Kinase Assay Kit are presented here. For additional information, refer to the product User Manual and web page.

Q1: What is the principle of this kit?

A1: Conventional measurement methods of PTK activity use radioactivity (^{32}P-ATP) and measure the amount of ^{32}P incorporated into synthesized peptide substrates after separating by electrophoresis, precipitation by trichloroacetic acid, or adsorption on a paper disk.

With the Universal Tyrosine Kinase Assay Kit, ATP and sample are added to a 96 well microtiter plate containing immobilized synthetic peptide substrates. PTK activity is measured by detection of peptide phosphorylation using an anti-phospho Tyrosine antibody labeled with Horseradish Peroxidase (HRP).

Q2: Can the activity of kinases other than PTK be measured using this kit?

A2: There are other Ser/Thr kinases, the synthetic peptide (Poly(Glu-Tyr)) and the antibody included in this kit are not appropriate for the measurement of the activity of other protein kinases.

Q3: Is there a possibility that Protein Tyrosine Phosphatase (PTP) could interfere with the measurement of PTK activity?

A3: PTP and Protein Phosphatase (PP) are present in cell extracts. To suppress these enzymes, Sodium Vanadate and NaF (PTP and PP inhibitors) have been added to the Extraction buffer and Kinase reacting solution.

Q4 : Is it possible that phosphorylated proteins derived from the sample extract could interfere with the measurement of PTK activity?

A4 : In this kit, the synthesized substrates are immobilized, so phosphorylated proteins derived from the sample are removed during the washing procedures. This results in a more accurate assay than kits that employ biotin-avidin to trap synthesized peptides. But in cases where the protein concentration of sample is very high, phosphorylated proteins may non-specifically bind to the plate. In this situation, we recommend that the sample be diluted or that the non-specific binding be assessed by using a non-treated 96-well microtiter plate as a blank.

Q5 : What kind of samples can be assayed with this kit?

A5: The kit is intended for the measurement of PTK activity in cultured cells or hematocyte components isolated from blood. When using tissue as a sample, we recommend the following procedure: mix tissue with Extraction buffer, then homogenized and spin. Use the supernatant directly in the assay.

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Q6: What is the specificity of synthesized peptide substrate that is used in the kit?

A6: The peptide substrate is Poly (Glu-Tyr) (4 : 1, molecular weight 20 - 50 kDa). This substrate is used for the measurement of the activity of various PTKs, such as FAK, ZAP-70, c-Src, and EGF-R. Other manufacturers offer kits containing synthesized substrates that are specific to each PTK, which increases the labor required (and, correspondingly, the potential for error) because a substrate specific to each target PTK must be prepared for each measurement. Takara Bio's Universal Tyrosine Kinase Assay Kit allows highly sensitive assay of the activity of target PTKs using a single non-radioactive substrate.

Q7: How is enzyme activity defined?

A7: Enzyme activity is based on the activity of recombinant c-Src. One unit (U) of enzyme is defined as the amount of enzyme needed to incorporate 1 pmol of phosphate into the substrate (KVEKIGEGTYGVVYK: 6 - 20 residue of p34cdc2) in 1 min.

Q8: What is the PTK control?

A8: The PTK control is a lyophilized crude extract of kinase prepared from cultured cells. It is used as a control in comparison to recombinant c-Src.

Q9: What is the sensitivity and measurement range of this kit?

A9: The detection sensitivity is 2.16 x 10^{-5} units/µl and the measurement range is from 2.16 -135 x 10^{-5} units/µl (86.4 - 5,400 x 10-5 units/well).

Q10: How many cells are needed to measure PTK activity?

A10: The number of cells required depends on the cell type and level of kinase activation. For example, for U937, P3U1, or Neuro2A cells that are cultured using standard methods, 500 - 1,000 cells are needed for detection.