MANUAL

MAb PT66-Eu cryptate

Part # 61T66KLA and 61T66KLB

Test size: 5,000 tests (61T66KLA), 20,000 tests (61T66KLB) - assay volume: 20 µL

Revision: #10 of September 2023

Store at 2-8°C

For research use only. Not for use in therapeutic or diagnostic procedures.

REAGENT DESCRIPTION

PT66 is a mouse monoclonal IgG1 which was produced using phosphotyrosine conjugated to BSA as immunogen. This monoclonal was shown to react specifically with phosphorylated tyrosine, both as free amino acid or when conjugated to carriers. No cross-reactivity has been observed with nonphosphorylated tyrosine, phosphothreonine, phosphoserine, AMP or ATP. It is suitable for use in a wide variety of tyrosine kinase activity tests involving receptor or non-receptor enzymes, and specific or ubiquitous substrates.

MATERIALS

Reagent	5,000 tests	20,000 tests
MAb PT66-Eu cryptate Lyophilized	1 vial	1 vial

Revvity reagents - Not provided	Part #
HTRF KinEASE detection buffer 200 mL - ready-to-use	62SDBRDF
Plates - HTRF 96-well low volume plate	66PL96001

For HTRF microplate recommendations, please visit *www.revvity.com*
For reading, an HTRF®-certified reader is needed. Make sure to use the setup for Eu^{3+} Cryptate.
For a list of HTRF®-certified readers and setup recommendations, please visit *www.revvity.com*

STORAGE AND STABILITY

- Store the lyophilized reagent at 2-8°C.
- Under appropriate storage conditions, reagents are stable until the expiry date indicated on the batch information.
- Upon reconstitution, the reagent stock solution is stable 1 week at 2-8°C. It can be refrozen (at ≤ -16°C) and thawed at least two more times.

ASSAY FORMAT

When used as suggested, one vial from the three available sizes will provide sufficient reagent for 5,000 tests, and 20,000 tests respectively using a 20 µL final assay volume.
Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.

	Volume
Other assay components	10 µL
Acceptor (d2 or XL665) conjugate	5 µL
Donor (Eu Cryptate) conjugate	5 µL
Final volume	20 µL

REAGENT HANDLING

BUFFERS

Revvity KinEASE detection buffer (#62SDBRDF) has been optimized for maximum performance and is ready to use.

When using specific in-house buffers for the preparation of working solutions, make sure to use **a phosphate-free buffer** (i.e. hepes), with a **pH maintained between 5.5 and 8.5**. It can be supplemented with BSA (0.1%), and detergents such as Tween 20, Triton X100 and CHAPS (up to 0.5%) to prevent reagent coating. Avoid SDS, due to its denaturing effect on XL665.

Use of Europium antibody conjugate solution requires a final KF concentration between 100 mM and 400 mM.

It is mandatory to use the same buffer to prepare the donor and the acceptor (d2 or XL665) conjugates.

CONJUGATES

Allow the lyophilized reagent to warm up at room temperature for at least 30 minutes.

Prepare the stock & working solutions according to the table below

MAb PT66-Eu cryptate*	Stock solution preparation	Working solution preparation (see assay format)
5,000 tests	Reconstitute with 250 µL distilled water. Mix gently.	Dilute 100-fold the stock solution in KinEASE detection buffer. Mix gently. E.g. Add 24.75 mL of KinEASE detection buffer to 0.25 mL of stock solution.
20,000 tests	Reconstitute with 1 mL distilled water. Mix gently.	Dilute 100-fold the stock solution in KinEASE detection buffer. Mix gently. E.g. Add 99 mL of KinEASE detection buffer to 1 mL of stock solution.

*Additional info is included on the batch information provided with the reagent

After reconstitution, the stock solution can be divided into aliquots and frozen for additional use.

Make sure to prepare stock and working solutions according to the instructions that correspond to the packaging you have purchased (number of tests).

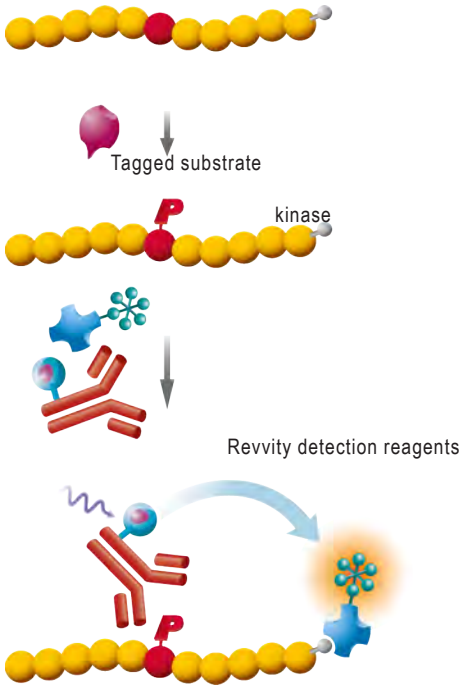
COMPANION REAGENTS

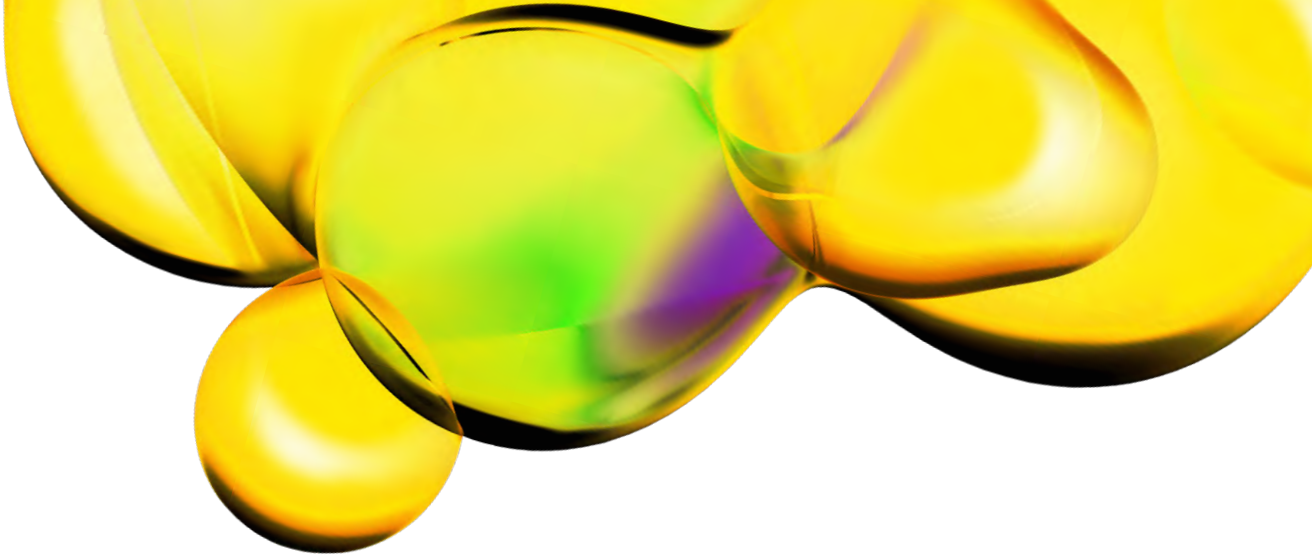
As illustrated beside, all kinase assays are based on the same format.

The enzymatic reaction is usually carried out with a biotinylated substrate (protein, peptide and the enzyme itself in the case of autophosphorylation).

The phosphorylated substrate is then detected using the specific anti phosphoresidue antibody coupled to Eu^{3+} Cryptate and a XL665 conjugate such as streptavidin-XL$^{ent!}$ (ref 611SAXLA) or Streptavidin-XL665 (ref 610SAXLA).

Alternatively, other tags such as GST-, 6HIS, c-myc or DNP may be used instead of biotin to label the kinase substrate.





Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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