MANUAL

# PAb Anti-phospho ATF2-Eu cryptate

**Part # 61P12KAE, 61P12KAZ and 61P12KAY**

**Test size: 500 tests (61P12KAE), 10,000 tests (61P12KAZ), 100,000 tests (61P12KAY)**

**Assay volume: 20 µL**

**Revision: #07 of September 2023**

**Store at ≤ -16°C**

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

## REAGENT DESCRIPTION

The Anti-Phospho ATF-2 (Thr71) antibody is a polyclonal antibody. It does not cross-react with the corresponding non-phosphorylated sequence. This antibody also recognizes both threonine 69 and 71 dually phosphorylated ATF-2, and threonine 71 singly phosphorylated ATF-2

## MATERIALS

| Reagent | 500 tests | 10,000 tests | 100,000 tests |
|---|---|---|---|
| PAb Anti-phospho ATF2-Eu cryptate.<br>Frozen, in Hepes pH 7.0 containing protease free BSA | 1 vial - 0.25 mL | 1 vial - 0.25 mL | 1 vial - 2.5 mL |

| Revvity reagents - Not provided | Part # |
|---|---|
| HTRF KinEASE detection buffer<br>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®- ompatible readers and setup recommendations, please visit *www.revvity.com*

## STORAGE AND STABILITY

- Store the reagents at ≤ -16°C.
- Under appropriate storage conditions, reagents are stable until the expiry date indicated on the batch information.
- Once thawed, stock solutions can be refrozen (≤ -16°C) and thawed once only. Do not repeat freezing and thawing.

## ASSAY FORMAT

When used as suggested, one vial from the three available sizes will provide sufficient reagent for 500 tests, 10,000 tests, and 100,000 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 stock solution to warm up at room temperature.

Prepare the conjugate solution according to the instructions included in the table below

| PAb Anti-phospho ATF2-Eu cryptate* | Stock solution | Working solution preparation (see assay format) |
|---|---|---|
| 500 tests | Ready to use<br>Mix gently | Dilute 10-fold the stock solution in KinEASE detection buffer. Mix gently.<br>E.g. Add 2.25 mL of KinEASE detection buffer to 0.25 mL of stock solution. |
| 10,000 tests | Ready to use<br>Mix gently | Dilute 200-fold the stock solution in KinEASE detection buffer. Mix gently.<br>E.g. Add 49.75 mL of KinEASE detection buffer to 0.25 mL of stock solution. |
| 100,000 tests | Ready to use<br>Mix gently | Dilute 200-fold the stock solution in KinEASE detection buffer. Mix gently.<br>E.g. Add 497.5 mL of KinEASE detection buffer to 2.5 mL of stock solution. |

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

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

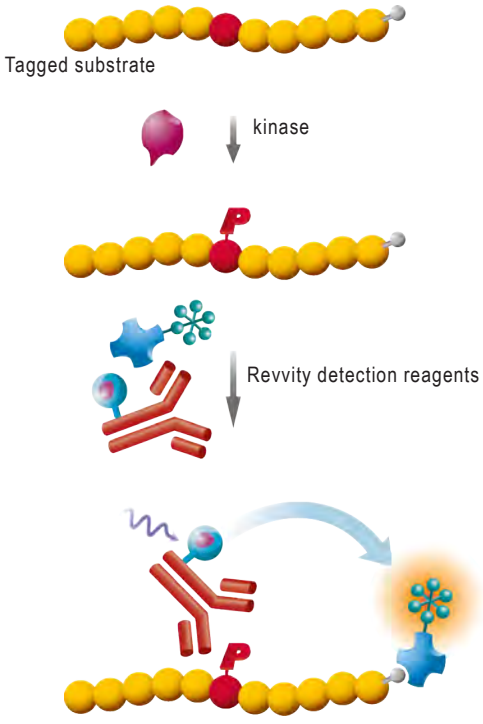
## COMPANION REAGENTS

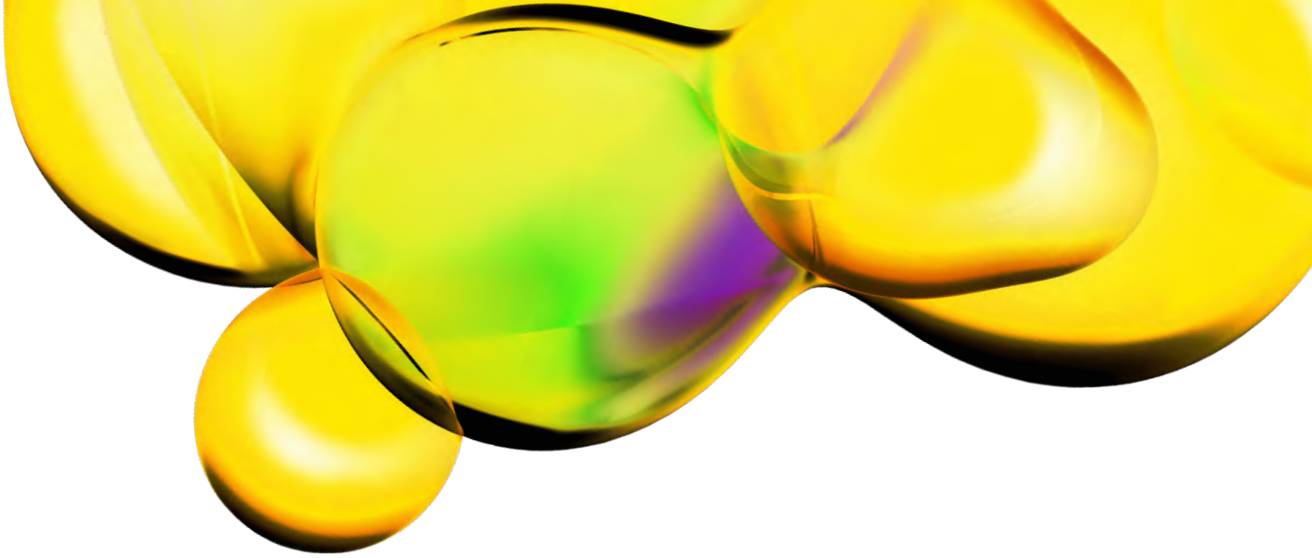
As illustrated beside, all kinase assays are based on the same format.

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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