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## Certificate of Analysis

### PI3 Kinase 4-Step Assay Reagent Kit (10000 wells)

Catalogue # 33-036

Lot # 1651936

**Product Description:** 10000 well kit of reagents for performing PI3 Kinase 4-step HTRF assays.

**Storage and Stability:** Stable for 12 months at -70°C from date of shipment.

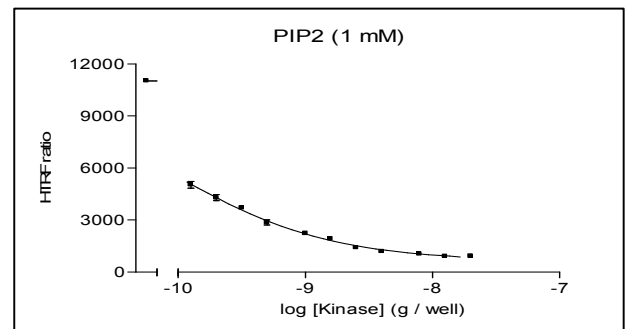
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material and store at -70°C.

**FOR IN VITRO RESEARCH USE ONLY  
 NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Reagent QC Assay:** This lot of PI3-K Reagents was used in a PI3-K assay in accordance with the protocol below. The Effective Concentration (EC) of kinase required to reduce the HTRF ratio by 60% and 85%.

EC (%)	60	85
[Kinase] (g/well)	1.67E-10	1.60E-9



**Reagent QC Assay:** This lot of PI3-K Reagents was successfully used to measure the APC counts in a PI3-K assay in accordance with the protocol below. Data shown is for no Kinase/PIP2 controls.

Mean APC Counts	26954
CV(%)	4

**Reagent QC Assay:** This lot of PI3-K Reagents was successfully used to measure the Eu Counts in a PI3-K assay in accordance with the protocol below. Data shown is for no Kinase/PIP2 controls.

Mean Eu Counts	25744
CV(%)	6

## Reagent QC Assay

### Preparation of Reagent QC Assay Solutions:

#### **Stop and Detection Component Preparation**

1. **Stop Solution:**

Prepare Stop Solution by combining Stop A and Stop B in a ratio of 3:1 respectively (e.g. to prepare Stop Solution for 36 wells combine 270µl of Stop A and 90µl of Stop B). This solution is stable at room temperature for up to 12 hours and should be prepared at least 2 hours prior to use.

2. **Detection Mix:**

Prepare the Detection Mix by combining DM C, DM B and DM A in a ratio of 18:1:1 respectively (e.g. to prepare Detection Mix for 36 wells combine 324µl of DM C, 18µl of DM B and 18µl of DM A, NB follow given order of addition.) This solution is stable at room temperature for up to 6 hours and should be prepared at least 2 hours prior to use.

#### **Reaction Component Preparation**

1. **1 x Reaction Buffer:** Dilute 4 x Reaction Buffer 4-fold with distilled water and add DTT to a concentration of 5mM. Prepare fresh for each assay. For 3ml of 1 x Reaction Buffer add 0.75ml of 4 x Reaction Buffer to 2.235ml of distilled water and 15µl of 1M DTT and mix. This solution is used to make up the ATP solution and the 1 x Reaction Buffer / PIP2 solution.

2. **ATP Working Solution:** Dilute the 10mM ATP Stock Solution in 1 x Reaction Buffer to 4 times the required final reaction concentration (e.g. to prepare 1ml of 100µM ATP final reaction concentration prepare a 400µM Working Solution by adding 40µl of 10mM ATP to 960µl of 1 x Reaction Buffer.)

3. **1 x Reaction Buffer / PIP2 Working Solution:** Dilute the 1mM PIP2 stock to a 13.8µM Working Solution in 1 x Reaction Buffer (this solution is 1.38 times the final assay concentration of 10µM.) For 2.008ml of 1 x Reaction Buffer / PIP2 solution, add 27.7µl of 1mM PIP2 to 1.980ml of 1 x Reaction Buffer and mix.

### Reagent QC Assay Procedure (384 well plate format):

For each Reagent QC Assay:

1. Mark up a black 384 well plate with a three by twelve well range with a silver or gold pen.
2. Add 0.5µl of 100% DMSO to each well.
3. On an eight by twelve Eppendorf rack, place twelve 1.5ml Eppendorf tubes in two columns of six.
4. To the first tube add 156µl of 1 x Reaction Buffer / PIP2 working solution and to the remaining eleven tubes add 72.5µl of 1 x Reaction Buffer / PIP2 working solution.
5. Dilute the test kinase to 10µg/ml in 1 x Reaction Buffer / PIP2 working solution with a volume greater than 50µl. This is the Primary dilution.
6. Add 25µl of Primary dilution to the first tube, vortex the tube and then titrate out kinase by transferring 108.8µl to the next tube in line except for the final tube.
7. Transfer 14.5µl of each kinase dilution in triplicate to the 384 well plate.
8. Add 5µl of ATP Working Solution to all wells.
9. Centrifuge the plate in a bench centrifuge for 1 minute.
10. Incubate the assay plate at room temperature for 30 minutes.
11. Add 5µl of Stop Solution to all wells.
12. Centrifuge the plate in a bench centrifuge for 1 minute.

13. Add 5µl of Detection Mix to all wells.
14. Centrifuge the plate in a bench centrifuge for 1 minute.
15. **(Note Stop Solution and Detection Mix must NOT be mixed prior to addition to the assay wells.)**
16. Incubate at room temperature for a minimum of 6 hours in the dark. It is recommended that the plate is sealed to minimise reduction in reaction volume. (The plate may be read 3 to 18 hours after the addition of the Detection Mix, protect from light during this time.)
17. Measure HTRF ratio on an appropriate reader according to the following parameters (these are guideline parameters, please also refer to parameters recommended in the instrument instruction manual):

Excitation	330 - 380nm
Emission	665 - 667.5nm and 620 - 635nm
Counting Delay	50µsec
Counting window	(integration time) 400µsec

Calculation:

HTRF Ratio is calculated as follows:

$$HTRF \text{ Ratio} = \left( \frac{Emission \text{ at } 665nm}{Emission \text{ at } 620nm} \right) \times 10000$$

Reviewed and approved by site quality representative.

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