

Validation of Redivue Tip adenosine 5'-[γ - 32 P]triphosphate in a Biotrak protein kinase C enzyme assay

Redivue Tip™ is a plastic pipette tip containing a radiolabeled nucleotide in a reversible and immobilized form. Here, we validate the use of Redivue Tip adenosine 5'-[γ - 32 P]triphosphate with the Biotrak™ protein kinase C enzyme assay system.

Using the pack leaflet for Biotrak protein kinase C enzyme assay system as a guide, we compared the phosphorylation rate of PKC [mixture of α , β , γ isoforms (Upstate cell signaling solutions, catalog number 14-115)] using both Redivue Tip adenosine 5'-[γ - 32 P]triphosphate and Redivue™ adenosine 5'-[γ - 32 P]triphosphate, triethylammonium salt.

Method

A dose response curve was prepared at 0, 1.25, 2.5, 5 and 10 ng of active PKC per tube and incubated with Redivue adenosine 5'-[γ - 32 P]triphosphate, triethylammonium salt and adenosine 5'-[γ - 32 P]triphosphate as contained in the Redivue Tip for 20 min at 37° C. The terminated mixtures (35 μ l each) were transferred to paper binding discs which were then washed for 2 \times 5 min in 5% (v/v) acetic acid. The discs were then transferred to 10 ml of scintillant and counted for 1 min on a β -scintillation counter. The experiment was repeated twice in duplicate.

Results

Redivue Tip adenosine 5'-[γ - 32 P]triphosphate and Redivue adenosine 5'-[γ - 32 P]triphosphate, triethylammonium salt produced similar results using the new Biotrak protein kinase C assay kit. The data shown here is representative of two different experiments (Fig 1).

Conclusion

Redivue Tip adenosine 5'-[γ - 32 P]triphosphate and Redivue adenosine 5'-[γ - 32 P]triphosphate, triethylammonium salt can be used interchangeably for protein kinase C determinations. We recommend using a buffer to elute the adenosine 5'-[γ - 32 P]triphosphate from the Redivue Tip prior to dilution with Magnesium [32 P]ATP buffer. All other steps in the assay are the same for both products.

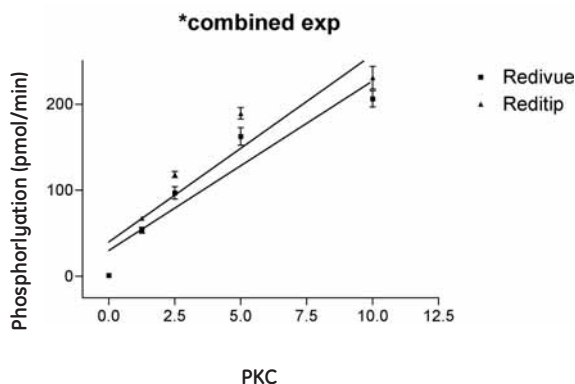


Fig 1. Dose response graph for PKC- α , β , γ using both Redivue Tip [γ - 32 P] ATP and Redivue [γ - 32 P] ATP.

Products Used

RT3001 GE Healthcare	Redivue Tip [γ - 32 P] ATP
AA0068 GE Healthcare	[γ - 32 P] ATP Redivue
RPN 77 GE Healthcare	Biotrak Protein Kinase C assay
14-115 Upstate Cell Signaling Solutions	PKC, active α , β , γ

Amount of PKC added	Redivue Tip	Redivue [γ - 32 P] ATP
	Total phosphate transferred (pmol/min)	Total phosphate transferred (pmol/min)
0 ng	0.3	0.8
1.25 ng	63	72
2.5 ng	114	115
5 ng	189	177
10 ng	227	232