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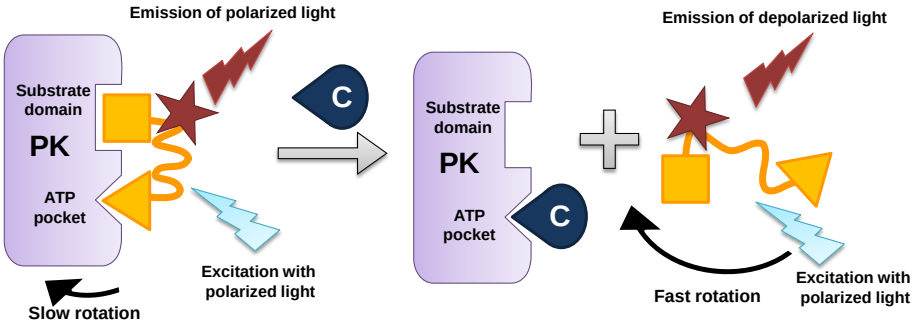
Product **ARC(CK2)-Fluo1: Protein Kinase CK2 Assay Kit from KINASERA OÜ**

Cat.No. PKAK-001-01

Description **ARC(CK2)-Fluo1 assay** is a fluorescence anisotropy(polarizarion)-based binding/displacement assay for the screening and characterization of inhibitors of protein kinase CK2. **ARC(CK2)-Fluo1 assay** is based on competitive displacement of a high-affinity fluorescent probe ARC(CK2)-Fluo1 from its complex with CK2. Thus the ARC(CK2)-Fluo1 assay is a binding assay (not a kinetic inhibition assay) [1].

The fluorescent probe ARC(CK2)-Fluo1 has very high affinity towards CK2α (K_D = 0.4 nM) [2], it is binding with high affinity to both the free CK2α catalytic subunit and CK2 holoenzyme. The unique bisubstrate character (simultaneous association with binding sites of both substrates of CK2) of the probe enables the characterization of inhibitors targeted to either ATP binding site or substrate protein/peptide binding domain of the kinase (Scheme 1).



Scheme 1. Binding of ARC(CK2)-Fluo1 probe (orange) to CK2 leads to high fluorescence anisotropy (polarization) value of the solution as the rotation of the probe is restricted in high molecular weight complex with the kinase and hence the emitted light is polarized. Displacement of ARC(CK2)-Fluo1 probe from the complex by a competitive inhibitor results in low anisotropy (polarization) value of the solution as ARC(CK2)-Fluo1 probe can freely rotate in solution and the emitted light is depolarized.

Kit contents
30 µL ARC(CK2)-Fluo1 probe (1 µM in DMSO)
2x1.5 mL 10X Assay Buffer
384-well Microtiter Plate with a Lid

Package size 1 kit

Storage condition 4 - 8 °C

Shipment conditions Room temperature

References

[1] A. Vaasa *et al.* High-affinity bisubstrate probe for fluorescence anisotropy binding/displacement assays with protein kinases PKA and ROCK. *Analytical Biochemistry*, (2009) **385**(1), 85-93.
[2] E. Enkvist *et al.* A subnanomolar fluorescent probe for protein kinase CK2 interaction studies. *Organic & Biomolecular Chemistry*, (2012) DOI: 10.1039/C2OB26022K.

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

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In vitro Kinase Assay

Assay Components

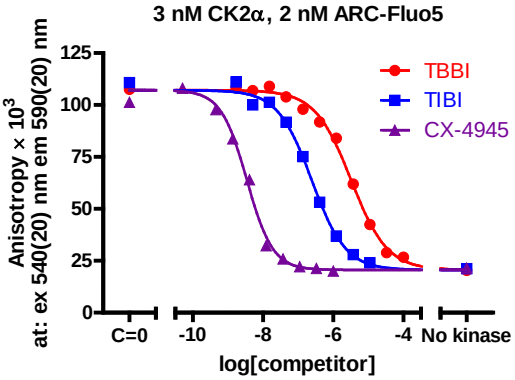
1X Assay Buffer (50 mM Hepes Buffer, 150 mM NaCl, 5 mM DTT, 0.005% w/w Tween-20, pH = 7.5)
ARC(CK2)-Fluo1 probe
Protein kinase: CK2alpha diluted in Assay Buffer directly before use
Test compounds

Assay Procedure

All compounds are pipetted into a well of a 384-well Microtiter Plate at room temperature

a. Prepare concentration series (three-fold dilutions; 13 different concentrations) of each inhibitor into a single column of a 384-well microplate in the volume of 16 µL.
   - The concentration of each sample should be 1.25-fold the desired concentration as 4 µL of ARC(CK2)-Fluo1 probe and kinase will be added later.
b. Dispense 16 µL of Assay Buffer into two wells
   - wells #N to #O
c. Dispense 20 µL of Assay Buffer in the last well of each column (wells #P); use these wells for the blank correction.
d. Prepare 5X complex of the kinase and ARC(CK2)-Fluo1 probe in Assay Buffer.
   - Use the optimal concentration of the kinase determined earlier (e.g., for the final concentration of the kinase of 3 nM and ARC(CK2)-Fluo1 of 2 nM prepare a solution of 15 nM kinase and 10 nM ARC(CK2)-Fluo1 probe),
   - the necessary volume of this solution is dependent on the number of inhibitors analyzed; 60 µL of this solution is enough for one inhibition curve.
e. Prepare 5X solution of ARC(CK2)-Fluo1 probe in Assay Buffer.
   - The necessary volume of this solution is dependent on the number of inhibitors analyzed; at least 4 µL of this solution is necessary for one inhibition curve.
f. Add 4 µL of the 5X complex of kinase and ARC(CK2)-Fluo1 probe to each well in rows (A-N) in the used columns. This will lead to the 20 µL final volume in the well for fluorescence measurements.
g. Add 4 µL of the 5X solution of ARC(CK2)-Fluo1 into wells in row O.
h. Incubate the plate on a thermostate for 15 minutes at 30°C and at constant rotation speed of 400 rpm.
i. Measure the anisotropy values. Use wells in the row P for blank correction. Use one well in row O for the adjustment of instrument settings.
j. Plot the obtained anisotropy values against log C (inhibitor). Analyze the curves using
k. sigmoidal dose-response (variable slope) function.

Representative data created at Kinasera



Displacement of fluorescent probe ARC(CK2)-Fluo1 (2 nM) from its complex with CK2α (3 nM) by various competitors:
CX-4945 (▲; 5-(3-chlorophenylamino) benzo[c] [2,6]naphthy ridine-8-carboxylic acid
TIBI (■; 4,5,6,7-tetraiodobenzimidazole)
TBBI (●; 4,5,6,7-tetrabromobenzimidazole)]