PKC Kinase Assay

ULight™-PKC Peptide and Europium-anti-phospho-PKC (Ala25Ser) Peptide Antibody

Two LANCE Ultra companion products - two convenient sizes!

ULight™-PKC peptide:
- TRF0108-D: 0.5 nmole, 1,000* assay points
- TRF0108-M: 5 nmole, 10,000* assay points
*0.5 pmol/assay point

PEPTIDE SEQUENCE:
CRFARKG$LRQKNV
Synthetic peptide derived from amino acids 19-31 of PKCα; phosphorylation site: Ser25.

VALIDATED FOR KINASES:
PKCα, PKCβ1, PKCβ2, PKCδ, PKCe, PKCγ, PKCη, PKCi, PKCθ, PKCζ

Europium-anti-phospho-PKC (Ala25Ser) Peptide Antibody:
- TRF0207-D: 10 µg, 1,562* assay points
- TRF0207-M: 100 µg, 15,625* assay points
*40 fmol/assay point

RECOGNIZED MOTIF:
RFARKG$pS$LRQKNV
Europium-labeled mouse monoclonal antibody recognizing phospho-Ser25 in human PKC in which Ala25 is mutated to Ser and phosphorylated.

LANCE Ultra Kinase Assays

LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W-1024 (Eu), with ULight, an innovative small molecular weight acceptor dye with a red-shifted fluorescent emission. In kinase assays, the binding of a Eu-labeled anti-phospho-substrate antibody to the phosphorylated ULight-labeled substrate brings donor and acceptor molecules into close proximity.

After irradiation of the kinase reaction at 320 or 340 nm, the energy from the Eu donor is transferred to the ULight acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of ULight-substrate phosphorylation.

Development of a PKC Kinase Assay

Additional reagents
PKC-α Invitrogen # P2227
LANCE Detection Buffer, 10X PerkinElmer # CR97-100
OptiPlate™-384, white PerkinElmer # 6007299
TopSeal™-A PerkinElmer # 6005185

Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl₂, 2 mM DTT and 0.01% Tween-20.
Suggested procedure

• Dilute the PKC-α kinase, ATP, inhibitors and ULight-PKCPeptide in Kinase Buffer.
• Prepare a 4X Detection Mix by diluting the Eu-anti-phospho PKC(Ala25Ser) antibody to 8 nM in 1X LANCE Detection Buffer.
• Add to the wells of a white OptiPlate-384:
  - 5 µL of PKC-α enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of ULight-PKC substrate/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
• Cover the plate with TopSeal-A and incubate for 30 min at room temperature (RT).

• Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
• Add 5 µL of 4X Detection Mix (Eu-anti-phospho-PKC(Ala25Ser) Antibody at a final concentration of 2 nM).
• Cover with TopSeal-A and incubate for 1 h at RT.
• Remove TopSeal-A and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 nm and emission at 665 nm).

NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course

PKC-α enzyme was incubated at concentrations ranging from 1 to 100 pM with 50 nM ULight-PKC substrate and 10 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration

Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 10 pM PKC-α and 50 nM ULight-PKC substrate. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve

Serial dilutions of staurosporine ranging from 30 pM to 3 µM (final concentrations in 1% DMSO) were incubated with 10 pM PKC-α, 50 nM ULight-PKC substrate and 10 µM ATP. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 4: Z’-factor Determination

PKC-α enzyme at 10 pM was incubated with 50 nM ULight-PKC substrate and 10 µM ATP with or without 1 µM staurosporine (final concentrations in 1% DMSO). Kinase reactions were terminated after 30 min by the addition of EDTA.