INSR Kinase Assay

ULight-IRS-1 (Tyr983) Peptide and Europium-anti-phospho-tyrosine Antibody (PT66)

Two LANCE Ultra companion products – two convenient sizes!

ULight™-IRS-1 (Tyr983) Peptide:
- TRF0120-D: 1 nmole, 1,000* assay points
- TRF0120-M: 10 nmoles, 10,000* assay points

*1 pmol/assay point

PEPTIDE SEQUENCE:
CKKSRGDYMTMQIG

Synthetic peptide derived from residues 979-989 of mouse Insulin receptor substrate 1 (IRS-1); phosphorylation site: Tyr983.

VALIDATED FOR KINASES: INSR, EphB4, FGFR1, FLT1, IGFR1, JAK1, JAK2, PDGFRα, RET, TIE2, TYK2

POTENTIAL SUBSTRATE FOR KINASES:
Tyrosine kinases specific for the YMxM motif

Europium-anti-phospho-tyrosine Antibody (PT66):
- AD0068: 50 µg, 7,500* assay points
- AD0069: 1 mg, 150,000* assay points

*40 fmol/assay point

RECOGNIZED MOTIF:
pTyr

Mouse monoclonal antibody directed against phospho-tyrosine.

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 an INSR Kinase Assay

Additional reagents

- INSR active Carna # 08-242
- 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 INSR kinase, ATP, inhibitors and ULight-IRS-1 (Tyr983) peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-tyrosine antibody (PT66) to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of INSR enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of ULight-IRS-1 (Tyr983) peptide/ ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A and incubate 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-tyrosine Antibody (PT66) 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.

These results show that LANCE Ultra reagents can be used to monitor the activity of kinases such as INSR with very high $K_m$ for ATP.