TECHNICAL NOTE

GSK3β Kinase Assay

ULight™ -Glycogen Synthase (Ser641/pSer657) Peptide &
Europium-anti-phospho-Glycogen Synthase (Ser641) Antibody

LANCE® *Ultra*

TECH NOTE *U*-TRF #34

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Two LANCE *Ultra* companion products – two convenient sizes!

ULight -GS (Ser641/pSer657) Peptide:

- TRF0131-D: 0.5 nmole, 1,000 assay points*
- TRF0131-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

PEPTIDE MOTIF:

PASVPPSPSLSRHSSPHQ(pS)ED

Synthetic peptide containing residues surrounding Ser641 of human Muscle Glycogen Synthase; this peptide is pre-phosphorylated on Ser657; phosphorylation site: Ser641.

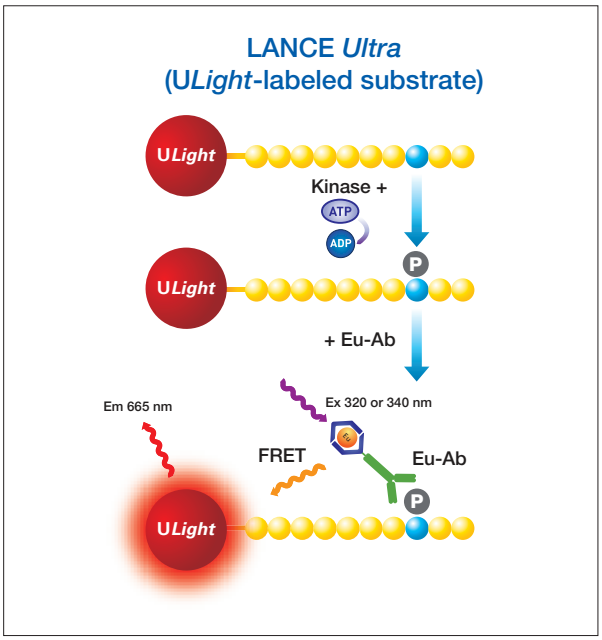
Europium-anti-phospho-GS (Ser641) Antibody:

- TRF0220-D: 10 µg, 1,562 assay points*
- TRF0220-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

RECOGNIZED MOTIF:

Europium-labeled rabbit polyclonal antibody recognizing human muscle glycogen synthase phosphorylated at Ser641.



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 U*Light*, an innovative small molecular weight acceptor dye with a red-shifted fluorescent emission. In kinase assays, the binding of an Eu-labeled anti-phospho-substrate antibody to the phosphorylated U*Light*-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 U*Light* acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of U*Light*-substrate phosphorylation.

PerkinElmer®
For the Better

Development of a GSK3β Kinase Assay

Additional Reagents:

GSK3β	Carna Biosciences #04- 141
LANCE® Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A	PerkinElmer # 6005185

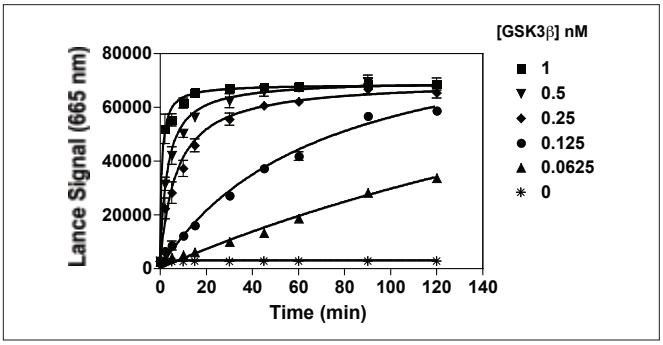
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20

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

Suggested Procedure

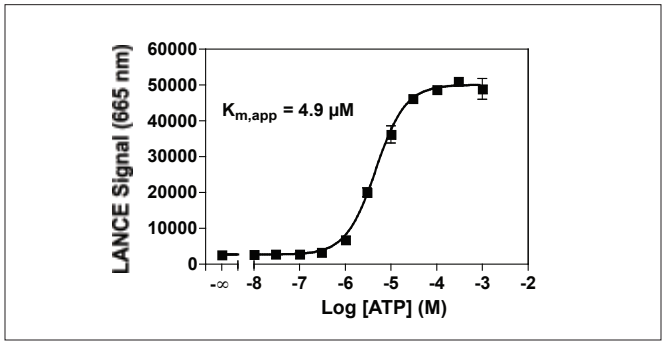
- Dilute the GSK3β enzyme, ATP, inhibitors and U*Light*-GS (Ser641/pSer657) Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-GS (Ser641) Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of GSK3β enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-GS (Ser641/pSer657) 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 24 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phospho-GS (Ser641) 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 & emission at 665 nm

Experiment 1: Enzyme Titration and Time-Course



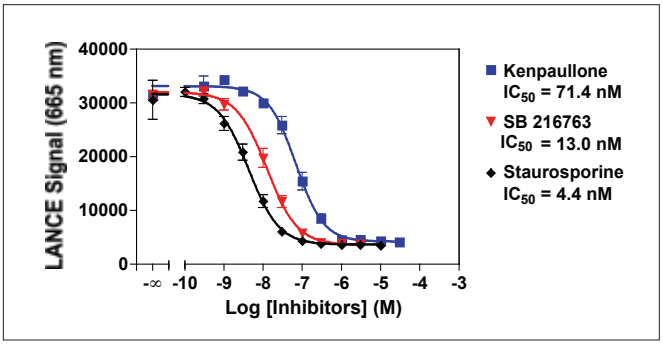
GSK3β enzyme was incubated at concentrations ranging from 62.5 pM to 1 nM with 50 nM U*Light*-GS (Ser641/pSer657) Peptide and 100 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA..

Experiment 2: ATP Titration



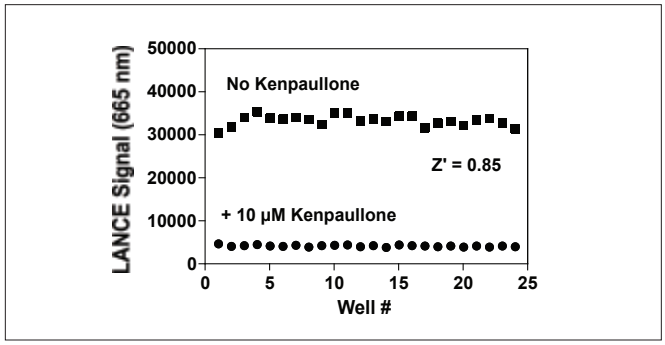
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 125 pM GSK3β enzyme and 50 nM of U*Light*-GS (Ser641/pSer657) Peptide. Kinase reactions were terminated after 45 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition



Serial dilutions of Kenpaullone ranging from 300 pM to 30 µM, serial dilutions of SB 216763 ranging from 300 pM to 3 µM and serial dilutions of staurosporine ranging from 100 pM to 10 µM (final concentrations in 2% DMSO) were incubated with 125 pM GSK3β enzyme, 50 nM U*Light*-GS (Ser641/pSer657) Peptide and 5 µM ATP. Kinase reactions were terminated after 45 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



GSK3β enzyme at 125 pM was incubated with 50 nM U*Light*- GS (Ser641/pSer657) Peptide and 5 µM ATP with or without 10 µM Kenpaullone (final concentrations in 2% DMSO). Kinase reactions were terminated after 45 min by the addition of EDTA

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