TECHNICAL NOTE

U-TRF #49

LANCE *Ultra* Tau-Ser400 *O*-GlcNAc hydrolase (OGA) Assay

LANCE® *Ultra* TR-FRET Technology

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This LANCE *Ultra* immunodetection assay measures the hydrolysis of an *O*-GlcNAc moiety from a biotinylated Tau-Ser400-*O*-GlcNAc peptide.

LANCE *Ultra* Europium-anti-*O*-linked-GlcNAc Antibody:

- TRF0413-D: 10 µg, 1,562 assay points*
- TRF0413-M: 100 µg, 15,625 assay points*

**6.7 fmol/assay point*

Peptide Sequence:

KWKHGAEIVYKSPVV-S(O-GlcNAc)-GDTSPRHLSNVK-K(biotin)-NH_2

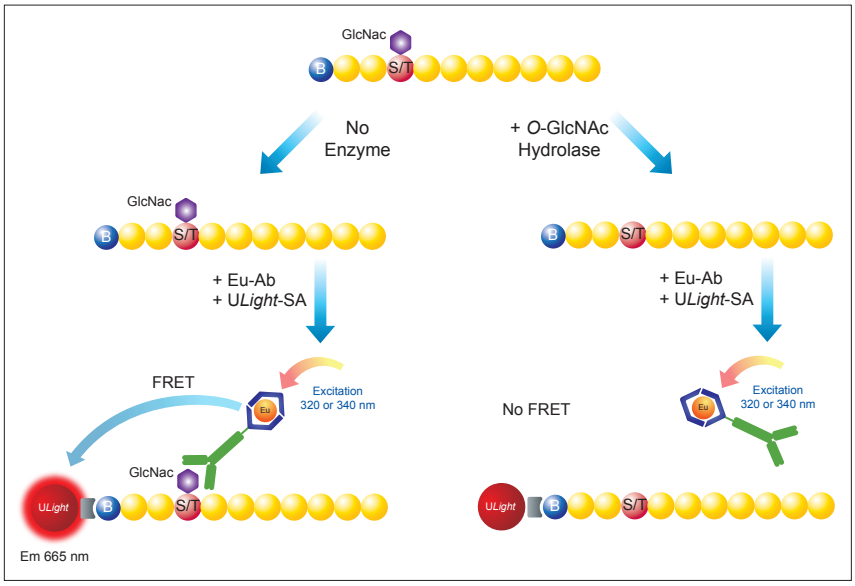

Figure 1. Schematic representation of the LANCE *Ultra* detection of an *O*-GlcNAcylated peptide (B: biotin group; S/T: serine or threonine residue).

LANCE *Ultra* Assays

LANCE *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W1024 (Eu), together with U*Light*™, a small molecular weight acceptor dye with a red-shifted fluorescent emission. In this technical note, we present the optimization of an OGA signal-decrease assay using as substrate a biotinylated Tau-derived peptide *O*-GlcNAcylated at Ser400. In this assay, detection of the non-hydrolyzed *O*-GlcNAcylated substrate was performed by the addition of the Eu-labeled antibody (Eu-Ab) and U*Light*-Streptavidin (U*Light*-SA), which bring the Eu donor and U*Light* acceptor dye molecules into close proximity. Upon irradiation 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 signal decrease is proportional to the activity of the OGA enzyme.

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Development of a LANCE *Ultra* Tau-Ser400 *O*-GlcNAc hydrolase (OGA) Assay

Reagents needed for this assay:

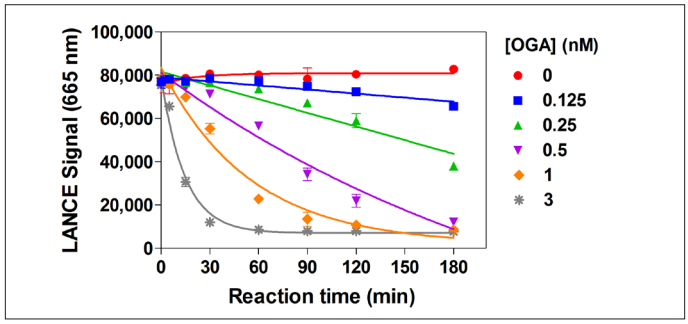
Europium-anti-*O*-linked-GlcNAc Antibody	PerkinElmer # TRF0413
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Tau-Ser400-*O*-GlcNAc (388-411), biotinylated	AnaSpec # 65409
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
O-GlcNAcase *(S. pyogenes),* recombinant (OGA)	Prozomix # PRO-E0255
White opaque OptiPlate™-384	PerkinElmer # 6007290
TopSeal™-A film	PerkinElmer # 6050195
O-(2-Acetamido-2-deoxy-D-glucopyranosylidene) amino N-phenyl carbamate (PUGNAc)	Carbosynth # EA06838
N6-Methyladenine	Carbosynth # FM10151

Assay buffer: 20 mM MES, pH 6, 0.05% BSA and 0.01% Tween

Standard Protocol

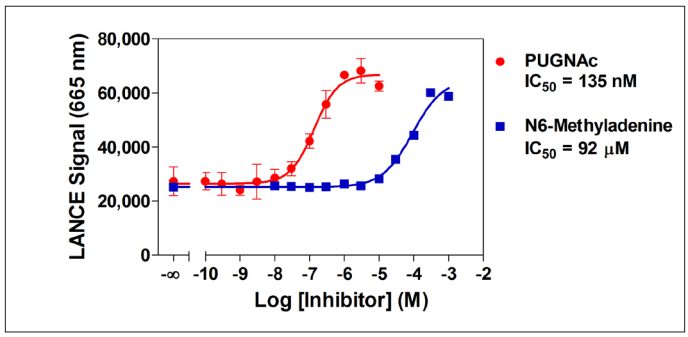
- Dilute OGA, inhibitors and biotinylated biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - Incubate for 5 min at room temperature (RT).
  - 5.0 µL biotinylated Tau-Ser400-*O*-GlcNAc peptide (2X)
- Cover the plate with TopSeal-A film and incubate at RT.
- Prepare Detection Mix by diluting the Eu-Ab to 0.67 nM and U*Light*-Streptavidin to 100 nM in 1X LANCE Detection Buffer (final concentrations of 0.33 nM and 50 nM in 20 µL total assay volume).
- Add 10 µL of Detection Mix. Addition of the Detection mix will stop the enzymatic reactions.
- Cover with TopSeal-A film and incubate 60 min at RT.
- Remove the TopSeal-A film and read signal with the EnVision® Multilabel Plate Reader in TR-FRET mode (excitation at 320 or 340 nm and emission at 665 nm).

Experiment 1: Enzymatic Titration and Time-Course



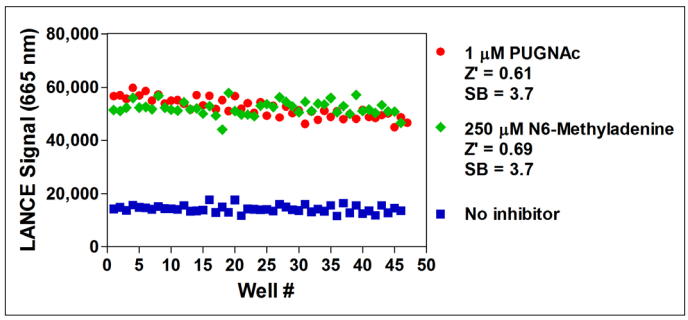
Enzymatic progress curves were performed by incubating OGA at concentrations ranging from 0.125 to 3 nM with 250 nM biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate. Detection Mix was added to stop the reactions at the indicated times and signal was read after 60 min. A 60 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: Enzyme Inhibition



Serial dilutions of PUGNAc and N6-Methyladenine ranging from 100 pM to 10 µM and 10 nM to 1 mM, respectively, were pre-incubated for 5 min with 1 nM OGA. Enzymatic reactions were initiated by the addition of 250 nM biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate. Enzymatic reactions contain 1% DMSO.

Experiment 3: Z'-factor Determination



OGA (1 nM) was pre-incubated with or without 1 µM PUGNAc or 250 µM N6-Methyladenine for 5 min. Enzymatic reactions were initiated by the addition of 250 nM biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate. Enzymatic reactions contain 1% DMSO.

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