TECHNICAL NOTE

AlphaLISA Tau-Ser400 *O*-GlcNAc hydrolase (OGA) Assay

AlphaLISA #28

AlphaLISA® Technology

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

Anti-*O*-linked-GlcNAc AlphaLISA Acceptor Beads

- AL144C: 250 µg; 500 assay points*
- AL144M: 5 mg, 10,000 assay points*
- AL144R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

Peptidic Substrate Sequence

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

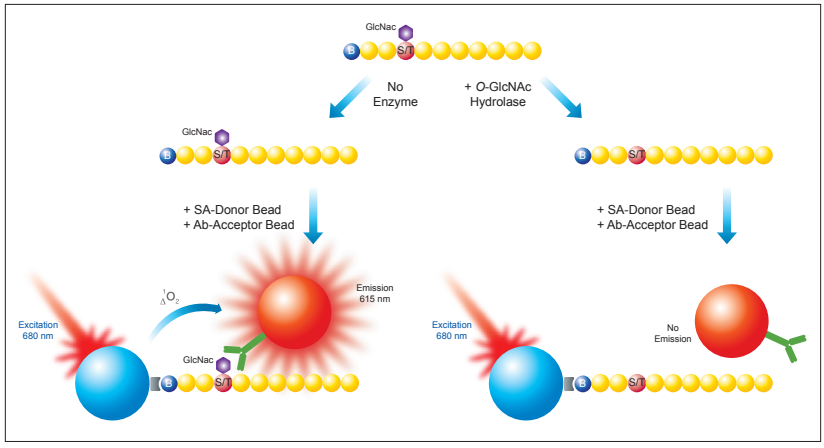

Figure 1. Schematic representation of the AlphaLISA detection of an *O*-GlcNAcylated peptide (B: biotin group; S/T: serine or threonine residue).

AlphaLISA Assays

AlphaLISA technology is a powerful and versatile platform that offers highly sensitive, no-wash immunoassays using Alpha Donor and AlphaLISA Acceptor beads. 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 Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the *O*-linked-GlcNAc moiety. Upon laser irradiation of the beads-target complexes at 680 nm, short-lived singlet oxygen molecules produced by the Donor beads can reach the Acceptor beads in proximity to generate an amplified chemiluminescent signal at 615 nm. The signal decrease is proportional to the activity of the OGA enzyme.

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Development of an AlphaLISA Tau-Ser400 *O*-GlcNAc hydrolase (OGA) Assay

Reagents needed for the assay:

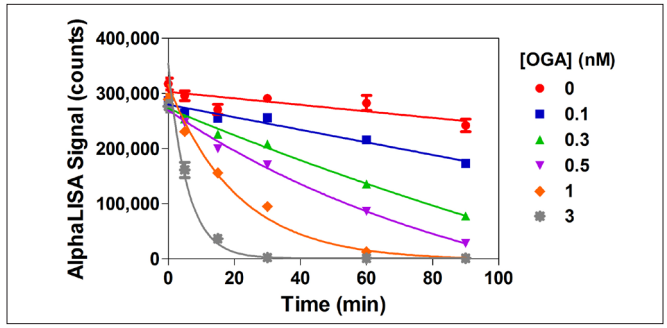
AlphaLISA anti-*O*-linked-GlcNAc Acceptor beads	PerkinElmer # AL144
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Tau-Ser400-*O*-GlcNAc (388-411), biotinylated	AnaSpec # 65409
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer # AL008
O-GlcNAcase *(S. pyogenes),* recombinant (OGA)	Prozomix # PRO-E0255
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 6.5, 0.05% BSA and 0.01% Tween

Standard Protocol

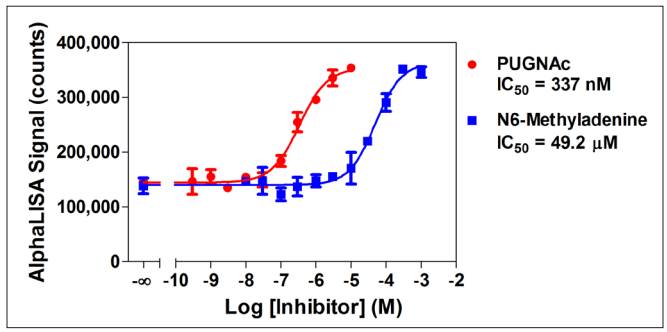
- Dilute OGA enzyme, inhibitors and biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - Incubate for 10 min at 23 °C.
  - 5 µL of biotinylated Tau-Ser400-*O*-GlcNAc peptide (2X)
- Cover the plate with TopSeal-A film and incubate at 37 °C.
- Prepare 1X Epigenetics Buffer 1 as recommended in the buffer technical data sheet.
- Prepare Acceptor beads at 100 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume).
- Add 5 µL of Acceptor beads. *Addition of Acceptor beads prepared in Epigenetics Buffer 1 stops the enzymatic reaction.*
- Cover with TopSeal-A film and incubate 60 min at 23 °C.
- Prepare Streptavidin Donor beads at 50 µg/mL in 1X Epigenetics Buffer 1 in subdued light (final concentration of 20 µg/mL in 25 µL total assay volume).
- Add 10 µL of Donor beads in subdued light.
- Cover with TopSeal-A film and incubate in subdued light for 30 min at 23 °C.
- Read signal in Alpha mode with the EnVision® or EnSpire® readers.

Experiment 1: Enzyme Titration and Time-Course



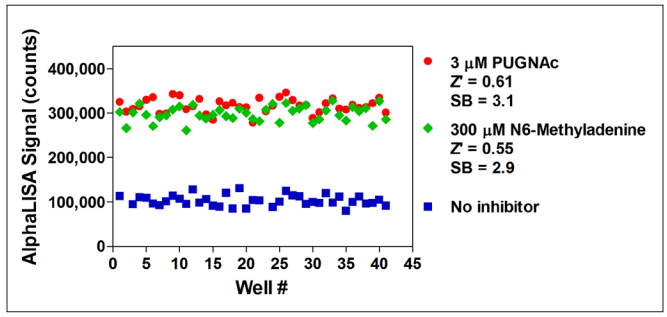
Enzymatic progress curves were performed by incubating OGA at concentrations ranging from 0.1 to 3 nM with 50 nM biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate. Enzymatic reactions contain 1% DMSO. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 30 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 300 pM to 10 µM and 10 nM to 1 mM, respectively, were pre-incubated for 10 min with 1 nM nM OGA. Enzymatic reactions were initiated by the addition of 50 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 3 µM PUGNAc or 300 µM N6-Methyladenine for 10 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated Tau-Ser400-*O*-GlcNAc peptide substrate. Enzymatic reactions contain 1% DMSO.

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