



TECHNICAL MANUAL

O-GlcNAcase (OGA) Activity Assay Kit

- **SKU CODE:** BR00012
- **SIZE:** 100 wells
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

O-GlcNAcase (OGA) Activity Assay Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

100 wells

Measuring instrument:

Microplate reader (405 nm)

Sample type:

Cell and tissue lysates, serum, plasma

2. Storage & Expiry

Store each component at the temperature indicated in Section 4 immediately on receipt. When stored as directed, kit components are stable for at least 12 months from receipt. Avoid repeated freeze-thaw cycles of frozen reagents; aliquot if multiple experiments are planned. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

O-linked β -N-acetylglucosamine (O-GlcNAc) is a dynamic sugar modification of serine and threonine residues on nuclear, cytoplasmic and mitochondrial proteins. Like phosphorylation, with which it extensively cross-talks, O-GlcNAcylation regulates protein function, signalling, transcription and nutrient sensing.

O-GlcNAc is added by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA, also known as NAG or MGEA5). Altered O-GlcNAc cycling is implicated in diabetes, cardiovascular disease, cancer and neurodegeneration.

Assay Principle: OGA in the sample hydrolyses the synthetic substrate p-nitrophenyl-N-acetyl- β -D-glucosaminide, releasing p-nitrophenol. Addition of NaOH stops the reaction and ionises p-nitrophenol to a yellow product measured at 405 nm. Coloured samples such as serum and plasma are run with a matched control to correct for background absorbance.

4. Kit Contents

No	Component Name	Size	Storage
1	OGA Assay Solution	10 ml (100 wells)	-20 °C
2	OGA Control Solution	10 ml	4 °C
3	10x Cell Lysis Solution	25 ml	Room temperature

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

1. Microplate reader capable of measuring absorbance at 405 nm
2. Clear, flat-bottom, uncoated 96-well plate
3. 1 N NaOH (stop solution)
4. 37 °C incubator (standard air incubator; do not use a CO₂ incubator)
5. Refrigerated microcentrifuge (~14,000 rpm)
6. Precision pipettes and tips
7. Deionised water (dH₂O)
8. Protein assay reagent (e.g. BCA)
9. Homogeniser for tissue samples

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We

advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. Use a standard 37 °C incubator; CO₂ incubators should not be used.
8. If lysates are turbid, add more 1x Cell Lysis Solution and re-pipette before centrifugation.
9. The Assay and Control Solutions contain acetate buffer; avoid contact with skin and eyes.
10. 1 N NaOH is corrosive; wear gloves and eye protection.

6. Reagent Preparation

1. **1x Cell Lysis Solution:** dilute 10x Cell Lysis Solution 1:10 with dH₂O.
2. Thaw the OGA Assay Solution and keep on ice during use. Keep the OGA Control Solution on ice when assaying coloured samples.
3. The 10x Cell Lysis Solution may precipitate when cold. Re-dissolve by warming briefly in a water bath with gentle agitation before use.

7. Sample Preparation

1. **Cells:** Resuspend $\sim 1 \times 10^5$ washed cells in 50–100 µl ice-cold 1x Cell Lysis Solution by gentle pipetting. Incubate on ice for 5 minutes with agitation.
2. **Tissue:** Homogenise ~ 5 mg tissue in 0.5 ml ice-cold 1x Cell Lysis Solution.
3. **Clarification:** Centrifuge at $\sim 14,000$ rpm for 5 minutes at 4 °C. Collect the supernatant and store at -80 °C. Recommended protein concentration: 0.5–4 mg/ml.
4. **Serum / plasma:** Use directly, following the coloured-sample procedure.

Store prepared samples at -80 °C and avoid repeated freeze-thaw cycles.

8. Assay Procedure

Bring all samples and reagents to the temperatures indicated before starting. Run each sample in duplicate where possible.

1. **Clear samples (lysates):** add 20 µl sample per well. Add 20 µl 1x Cell Lysis Solution or dH₂O to a separate well as the blank.
2. Add 100 µl OGA Assay Solution to each well and mix briefly.
3. Cover and incubate at 37 °C for 30 or 60 minutes.
4. Add 20 µl 1 N NaOH to each well and mix briefly.
5. Wait 3 minutes, then read absorbance at 405 nm.
6. **Coloured samples (serum/plasma):** add 10 µl sample to two wells. Add 100 µl OGA Control Solution to one well and 100 µl OGA Assay Solution to the other, then incubate, stop and read as above.

9. Data Analysis

Calculation:

Clear samples: $\Delta OD = OD_{405} \text{ (sample)} - OD_{405} \text{ (blank)}$

Activity (IU/L) = $\Delta OD \times 1000 \times 140 / (t \times 0.6 \times 18 \times 20)$ — 30 min: **$\Delta OD \times 21.6$** ; 60 min: **$\Delta OD \times 10.8$**

Coloured samples: $\Delta OD = OD_{405} \text{ (assay well)} - OD_{405} \text{ (control well)}$

Activity (IU/L) = $\Delta OD \times 1000 \times 130 / (t \times 0.6 \times 18 \times 10)$ — 30 min: **$\Delta OD \times 40.12$** ; 60 min: **$\Delta OD \times 20.06$**

One unit (IU) is defined as the amount of enzyme that converts 1 μmol of substrate per minute under the assay conditions.

Note:

t: incubation time (min).

140 / 130: total volume per well in μl (clear samples: 20 μl sample + 100 μl OGA Assay Solution + 20 μl 1 N NaOH; coloured samples: 10 μl sample + 100 μl solution + 20 μl 1 N NaOH).

0.6: light path length (cm).

18: extinction coefficient of p-nitrophenol at 405 nm ($\text{mM}^{-1}\text{cm}^{-1}$).

20 / 10: sample volume in μl (clear / coloured samples).

1000: conversion factor from mmol to μmol .

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

