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Endoglycosidase H digestion of GCase

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The N-linked glycoprotein profile of GCase trapped in the ER makes it susceptible to digestion by the glycosidase EndoH. Western blotting of GCase following EndoH digestion will yield an additional lower molecular weight band that corresponds to the GCase that is trapped in the ER.

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Principle

1 To study the subcellular localisation and transport of GCase mutants, the processing of its *N*-linked glycans was monitored by performing Endo H F digestions. Endo H cleaves only high mannose structures and hybrid structures.

The N-linked glycoprotein profile of GCase trapped in the ER makes it susceptible to digestion by the glycosidase EndoH. Western blotting of GCase following EndoH digestion will yield an additional lower molecular weight band that corresponds to the GCase that is trapped in the ER.

Method

2 Harvest cells (at least 6 well plate for SH/60 mm plate for MEFs, fibroblasts) by trypsinisation. Pellet cells at 200 xg. Wash once in 1 ml PBS. Pellets can be frozen or continue straight on to digestion.

3 Lyse cells in 1% (v/v) TX-100 in PBS supplemented with protease inhibitors (1 mM PMSF, 1 mg/ml leupeptin, 1 mg/ml pepstatin A). Lysates need to be at least 2 mg/ml. Therefore lyse 6-well in 100 ml. Incubate on ice, 15 minutes. Pellet debris at full speed in microfuge, 10 min, 4 °C.

NB. Protease inhibitors toxic. Always wear gloves. Weigh out stocks in fume hood.

4 Measure protein concentration with BCA kit.

5 EndoH enzyme and buffers is from New England Biolabs (Cat# P0702L).

Kit contains:
50,000 units EndoH (500 units/ml)
10X Glycoprotein Denaturing Buffer
10X G5 reaction buffer

6 For robust detection of GCase after endoH treatment require 10 (minimum) to 20 µg of protein (maximum) per reaction.

For very low protein concentrations e.g L444P/L444P lines, up to 70 µg protein can be used in order to visualise bands easily by western blot.

7 For each sample there are two treatments: (1) without Endo H (2) with Endo H

In small eppendorf mix:
9 µl sample (10-20 mg) protein*
1 µl 10X Glycoprotein Denaturing buffer

* dilute sample accordingly in H_20

8 Heat sample in block at 100 °C for 10 minutes (make sure tubes are tightly sealed otherwise will pop open and sample evaporates).

9 Briefly centrifuge tubes to collect reaction at bottom of tube.

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10 To each tube containing denatured sample add 10 ml of reaction mixture:

	Without EndoH	With EndoH
10X G5 reaction buffer	2 ml	2 ml
EndoH (1000 units)	0 ml	2 ml
H20	8 ml	6 ml

For multiple samples it is best to make a master mix.

11 Incubate at 37 °C for 1 hour (can be up to 3 hours).

12 Stop reaction by adding 5 µl of NuPAGE 4X gel loading buffer and 1 µl 10X reducing agent. Proceed to western blot or freeze samples at -20 °C.

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