# PROTOCOL FOR ENZYMATIC DEGLYCOSYLATION

## Usual deglycosylating enzymes:

**- chondroitinase ABC (CH):** Removes chondroitin sulfate of the types A, B and C from proteoglycans. Best source: Seikagaku (Associates of Cape Cod). Use CH protease-free to avoid protein damage during dechondroitinization (10 U/ml). Alternative sources: MP-Biologicals and Sigma-Aldrich

**- O-glycosidase (OG):** Removes O-linked carbohydrates of the type Core-I from glycoproteins. It does not work well if the samples are not treated simultaneously with sialidase. Source: Roche Applied Science (500 U/ml)

**- N-glycosidase-F (NG):** A.k.a *PNGase-F*. Removes N-linked carbohydrates bound to Asn. It may need previous denaturation of the samples. Source: New England Biolabs (500 kU/ml)

**- Sialidase (Si):** A.k.a. *Neuraminidase*. Removes sialic acid from N-linked or O-linked carbohydrates. Source: Roche Applied Science (10 U/ml)

## Usual buffers for enzymatic deglycosylation:

5x CH buffer: 200 mM TrisHCl + 200 mM sodium acetate, pH 8.0

16X DG buffer: 400 mM Sodium phosphate mono/dibasic, pH 7.4 (see our buffers list). This buffer is compatible with most glycosydases but the enzymes will lose activity over time. Chondroitinase ABC will not work well in this buffer in complete absence of acetate (you may add sodium acetate to a final concentration of 10 mM to regain CHase activity)

The following reactions are calculated for a total volume ≤50 µl. The volume can be adjusted depending on the total amount of protein in the sample, to avoid sample dilution.

For examples of the effects of these enzymatic reactions on the SDS-PAGE profile of a proteoglycan (brevican) from native tissue, see Viapiano et. al. (J. Biol. Chem. 2005) and Viapiano et. al. (Cancer Res. 2005).

Protease inhibitors: *Complete, EDTA-free* (Roche Applied Science)

## De-chondroitinization:

Removes only chondroitin/dermatan sulfate

- sample x µl (up to 50-100 µg total protein)
- 5X CH buffer 10 µl
- 50X protease inhibitors 1 µl
- 500 mM EDTA 1 µl (this is optional and **cannot be used** if the samples are going to be tested for metalloprotease activity)
- Chondroitinase ABC 0.5 µl (increase to 1 µl if the sample is > 50 ug total protein)
- milliQ H2O 37.5-x µl

Incubate at 37C for 4-8 hours (use 8h for highly GAGylated proteoglycans like versican or aggrecan, and 4h for smaller proteoglycans like brevican)

**"Complete" deglycosylation:**
Removes exposed N-linked sugars and type-I core O-linked sugars. It will not remove buried N-linked sugars, O-linked sugars with other core types, or non-conventional glycosylation (e.g., O-linked fucosylation and mannosylation)

| | |
|---|---|
| - sample | x µl (up to 50-100 ug total protein) |
| - 16X DG buffer | 3 µl |
| - 0.5 M sodium acetate (pH 7) | 1 µl (optional, add only if CH enzyme is used) |
| - 50X protease inhibitors | 1 µl |
| - Chondroitinase ABC | 0.5 µl (increase to 1 µl if the sample is > 50 ug total protein) |
| - O-glycosidase | 0.5 µl (idem) |
| - Sialidase | 0.5 µl (idem) |
| - N-glycosidase F | 0.5 µl (idem) |
| - milliQ $H_2O$ | 43-x µl |

Incubate at 37C for 8 hours

**Denaturing deglycosylation for N-linked carbohydrates:**
It will remove all N-linked carbohydrates.

Samples need to have a high protein concentration (>1 mg/ml).

a) Denaturation of 30 µl of sample (≥30 ug total protein).

| | |
|---|---|
| - sample (culture medium or lysate **without detergents**) | 30 µl |
| - 1% w/v SDS (dilute 20% stock SDS 1/20 with milliQ $H_2O$) | 3.5 µl |
| - 2.5 M beta-mercaptoethanol (dilute pure BME 1/6 with milliQ $H_2O$) | 1.5 µl |

(final conditions: 35 µl reaction with 0.1% SDS and 0.1M BME)

b) Incubate the samples at 95C for 5 min and then cool down to 4C.

c) Deglycosylation mixture:

| | |
|---|---|
| - denatured sample | 35 µl (up to 50-100 ug total protein) |
| - 5% v/v NP-40 detergent | 8 µl |
| - 16X DG buffer | 3 µl |
| - 50X protease inhibitors | 1 µl |
| - N-glycosidase F | 0.5 µl (increase to 1 µl if the sample is > 50 ug total protein) |
| - milliQ $H_2O$ | 2.5 µl |

Vortex well before adding N-glycosidase. Incubate at 37C for 4 hours.