Description: Endo S PRIME-LY™ is an endoglycosidase with a uniquely high specificity for cleaving the N-linked glycans from the chitobiose core of the heavy chain of native IgG. This enzyme will leave a single N-Acetylglucosamine, with or without an attached fucose molecule, attached to the IgG. Endo S PRIME-LY™ is a recombinant glycosidase cloned from *Streptococcus pyogenes*.

Biological Source: E. *coli*.

Concentration: 200,000 U/mL.

Molecular Weight: Endo S PRIME-LY™ has a molecular weight of approximately 108kDa

Physical Form: Endo S PRIME-LY™ is supplied as a lyophilized powder. When reconstituted, the resulting solution will be 20mM Tris-HCl, 50mM NaCl and 5mM EDTA at a concentration of 200,000 (U/mL).

Storage Conditions: Endo S PRIME-LY™ is stable at RT and can be stored at RT upon receipt.
Endo S PRIME-LY™-50 is reconstituted by adding 50µL ddH_2O and vortexed.
Endo S PRIME-LY™-100 is reconstituted by adding 100µL ddH2O and vortexed.
After reconstitution, the enzyme is stable for 1 month at 4°C but should be kept at -20° to -80°C for long term storage. After reconstitution, avoid freeze-thaw cycles. The 10X Endo S Reaction Buffer can be stored at RT.

Unit Definition: One unit of reconstituted Endo S PRIME-LY^{TM-} will catalyze the deglycosylation of 10 µg of IgG in 60 minutes at 37^{o}C. One unit is equal to 1 IUB milliunit.

This lot passes the following Quality Control specifications:

- **Activity Assay:** Native human IgG (100µg) is incubated with reconstituted Endo S PRIME-LY^{TM} -50 for 60 minutes at 37°C, and then analyzed by SDS- PAGE. Fully glycosylated IgG heavy chain migrates at approximately 50kDa. Deglycosylation is assessed by the presence of deglycosylated IgG heavy chain with an apparent molecular weight of 47kDa following staining via Coomassie Brilliant Blue™.

Purity: ≥95% as determined by SDS-PAGE analysis and staining with Coomassie Brilliant Blue™.

Protein Deglycosylation Using Reconstituted Recombinant Endo S PRIME-LY™

Note: The following protocols are intended as a general guide for IgG deglycosylation and may require modification for different antibody substrates. Like many enzyme reactions, it is highly dependent on reaction conditions and should be determined empirically for each target.

General Protocol for the deglycosylation of human IgG under Native Conditions

Materials to Be Supplied By the User:

- Double distilled or other high quality Mass Spectrometry grade water.
  a. Add up to 100µg of the target IgG in water (or a compatible buffer at a low ionic strength) to a final volume of 17µl.
  b. Add 2 µl of the 10X Endo S Reaction Buffer (supplied – see below).
  c. Add 1µl of reconstituted Endo S PRIME-LY™
  d. Incubate at 37°C for 30-60 minutes.

Note: Deglycosylation of glycoproteins may be visualized by gel-shift on SDS-PAGE, with the deglycosylated product running faster than the glycosylated substrate. This enzyme will leave a single N-Acetylglucosamine, with or without an attached fucose molecule, attached to the IgG.

10X Endo S Reaction Buffer:

- 50 mM $CaCl_2$,
- 500mM sodium acetate, pH 5.5