Biotinylation of RPL

Please note that below is only a general guide based on methodology of a successful research project that used GlycoSeLect's RPL. You can try other biotinylation kit that you prefer and follow their manufacturer instructions.

Biotinylation of recombinant prokaryotic lectins (RPLs)

GlycoSeLect RPLs were biotinylated using EZ-Link NHS-PEG4-Biotin (ThermoFisher Scientific, product No. A39259). Biotinylation was performed in PBS solution according to manufacturer instruction. Although the recommendation for amount of Biotin was using 20-fold excess of targeted protein at 2mg/mL, in this experiment, amount of biotin was 4-fold molar excess of RPL, and it was sufficient for using in our experiment. Then the mixture of biotin and RPL was incubated on ice for 2 hours. The excess of biotin was removed using Zeba™ Spin Desalting Columns (ThermoFisher Scientific 89889 & 89894). Biotinylated RPLs were stored at -80 °C.

Notes:

1. If RPLs are going to be used in short term (within a month), can be stored at -20 °C.
2. It is better to aliquot the RPLs before storing to prevent a negative freeze/thaw effect.
3. RPL activity needs presence of metal ions therefore all buffers must be supplemented with 1 mM $CaCl_2$, 1 mM $mgCl_2$ and 1mM $MnCl_2$ prior to using the biotinylated RPL. The recommended working buffer is Tris buffered saline (TBS), pH 7.6.
4. Following dilution with TBS, supplemented with the metal ions, the RPL should be incubated for a minimum 30 minutes prior to use to allow metal complexation.

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