# Affinity Purification of 20 kDa mPEG-BSA using 9B5 Anti-PEG Agarose

## Product Description

9B5 Anti-PEG agarose (Cat No 9B5-AG) was prepared by irreversibly coupling monoclonal antibody 9B5-6-25-7 to agarose via its FC region. Approximately 0.6 mg of antibody was coupled per ml of settled agarose.

## Methods

1. 9B5 Anti-PEG agarose (3 ml) was equilibrated with 150 mM NaCl, 10 mM sodium phosphate, 0.1% $NaN_3$ pH 7.2 (PBS) in a 1 cm i.d. column.
2. 1.0 ml of a mixture containing 0.125 mg of 90% pure 20 kDa mPEG-BSA and 0.5 mg of human myoglobin in PBS was applied to the column.
3. The column was washed with 4 x 2 ml of PBS.
4. Elution was performed with 9 x 1 ml of 25% ethylene glycol, 1% PEG-8000 in 50% PBS.
5. Fractions were analyzed by SDS PAGE on a 12.5% Laemmli mini-gel.

## Results

As shown below, 20 kDa mPEG-BSA binds to 9B5 Anti-PEG agarose, whereas non PEGylated proteins including BSA and myoglobin pass through the column. Pure 20 kDa mPEG-BSA is eluted using a buffer containing 25% ethylene glycol and 1% PEG-8000.

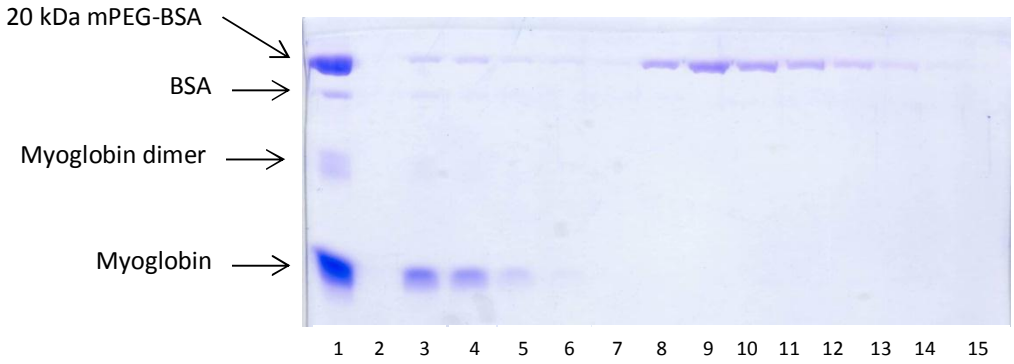


Fig.1. Coomassie Blue stained SDS PAGE gel illustrating 9B5 Anti-PEG affinity purification of 20 kDa mPEG-BSA. Lane 1, column load. Lanes 2-6, flow-through and wash fractions. Lanes 7-9, elution fractions.

## Comments

1. Samples must be free of unconjugated PEG prior to application to anti-PEG agarose
2. PEGylated protein can be eluted using 25% ethylene glycol without PEG-8000 but elution will require a larger volume of buffer. Ethylene glycol can subsequently be easily removed by dialysis.
3. 9B5 Anti-PEG agarose can be regenerated by washing with 25% ethylene glycol in PBS (~20 column volumes) followed by re-equilibration in PBS.

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