

## Application Note #10

### Coupling of proteins to CL-30Q02-6C, Carboxyl coated Molday ION

*BioPAL also provides a best efforts service to couple your protein to CL-30Q02-6C following this protocol for a minimum custom price of \$2000.*

Carboxyl coated Molday ION can be coupled with proteins to form amide bonds. This enables the immobilization of proteins, peptides and antibodies.

Proteins can be coupled to CL-30Q02-6C by following this protocol.

- Dialyze 1ml CL-30Q02-6C twice with MES buffer.
- Add EDC solution. (0.4 ml/ml CL-30Q02-6C)
- Add NHS solution (0.6 ml/ml CL-30Q02-6C)
- Incubate for 20 min at room temperature under rotation.
- Pass the activated CL-30Q02-6C over a NAP-G-25 column equilibrated with MES buffer to remove unreacted reagent following directions of the column manufacturer.
- Drip the activated CL-30Q02-6C into a stirring solution of protein dissolved in 0.1M sodium bicarbonate buffer (pH 8.0) at a concentration of 1 mg ligand per ml CL-30Q02-6C.
- Incubate for 120 min at room temperature.
- Ultrafilter coupled CL-30Q02-6C using a 100K MWCO Pall membrane 3 times against PBS to separate unreacted protein from coupled CL-30Q02-6C.

**The following reagents may be purchased from BioPAL or prepared by each investigator.**

<b>MES buffer (200ml)</b> Dissolve 1.06 g MES (2-(4-Morpholino)ethanesulphonic acid hydrate) in 90 ml H <sub>2</sub> O, adjust pH to 5.5, fill with H <sub>2</sub> O to 100 ml.	<b>BioPAL Catalog No. MJ0001</b>	<b>Price \$65.00</b>
<b>EDC solution (20mg)</b> Dissolve 10 mg/ml EDC (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) in MES buffer	<b>BioPAL Catalog No. MJ0002</b>	<b>Price \$65.00</b>
<b>NHS solution (20mg)</b> Dissolve 10 mg/ml NHS (N-Hydroxysuccinimide) in MES buffer	<b>BioPAL Catalog No. MJ0003</b>	<b>Price \$65.00</b>
<b>Carbonate buffer (100ml)</b> Dissolve 0.84 g sodium bicarbonate in 90 ml H <sub>2</sub> O, adjust pH to 8.0, fill with H <sub>2</sub> O to 100 ml.	<b>BioPAL Catalog No. MJ0004</b>	<b>Price \$65.00</b>
<b>PBS (200ml)</b> Dissolve 0.26 g NaH <sub>2</sub> PO <sub>4</sub> H <sub>2</sub> O (Sodium phosphate monobasic monohydrate) + 1.44 g Na <sub>2</sub> HPO <sub>4</sub> 2H <sub>2</sub> O (Sodium phosphate dibasic dehydrate) + 8.8 g NaCl (Sodium chloride in 900 ml H <sub>2</sub> O, adjust pH to 7.4 and fill to 1000 ml	<b>BioPAL Catalog No. MJ0005</b>	<b>Price \$65.00</b>
<b>NAP column (2 columns)</b> May be purchased from GE Healthcare	<b>BioPAL Catalog No. MJ0006</b>	<b>Price \$65.00</b>
<b>Pall filter (2 filters)</b> VWR catalog No. 29300-046	<b>BioPAL Catalog No. MJ0007</b>	<b>Price \$65.00</b>
<b>Ultrafiltration device</b> May be purchased from Millipore		