Red Sepharose® CL-6B *INSTRUCTIONS*

Red Sepharose CL-6B is Procion®Red HE-3B covalently attached to Sepharose CL-6B by the triazine coupling method. The immobilized red dye will bind to a wide variety of $NADP^+$- and NAD^+-dependent enzymes, including many kinases, dehydrogenases and transferases. Carboxypeptidase G, interferon, plasminogen and plasminogen activator are examples of other proteins that have been purified on Red Sepharose CL-6B. The cross-linked matrix provides a stable, rigid gel even in the presence of dissociating agents.

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Edition AC

Table 1. Gel characteristics.

Ligand density:	1.7 -2.3 μmole Procion Red/ml drained gel
Available capacity:	ca 2 mg Rabbit muscle LDH/ml drained gel
Bead structure:	6% cross-linked agarose
Bead size range:	45-165 μm
Mean bead size:	90 μm
Max linear flow rate*:	150 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability**	
Long term:	4-12
Short term:	3-13
Chemical stability:	Stable to all commonly used aqueous buffers and additives such as 8 M urea and 6 M Guanidine hydrochloride
Physical stability:	Negligible volume variation due to changes in pH or ionic strength
Autoclavable:	At 121 °C for 30 min

* Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

** The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration and cleaning.

Preparing the gel

Red Sepharose CL-6B is supplied freeze-dried (1 g freeze-dried gel gives about 4 ml swollen gel). Swell the required amount of the freeze-dried gel for approximately 15 minutes in distilled water. Wash the swollen gel on a sintered glass filter with about 200 ml distilled water per gram freeze-dried gel, added in several aliquots. This will remove the additives used to preserve the swelling

properties of the gel. Prepare a slurry with binding buffer in a ratio of 75% settled gel to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

Packing Sepharose CL-6B gels

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the gel slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see Table 1, is typically employed during packing.

   Note: If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adaptor

Adaptors should be fitted as follows:

1. After the gel has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.

3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump, and column and the sample application system (LV-3 or LV-4).
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the gelsurface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the gel bed is stable. Re-position the adaptor on the gel surface as necessary.

The column is now packed and equilibrated and ready for use.

Binding

Proteins which specifically bind to Red Sepharose CL-6B do so around neutral pH. Different substances differ in their affinity for Red Sepharose CL-6B. The available capacity will depend upon parameters such as sample concentration, flow rate, pH, buffer composition and temperature. To obtain an optimal purification with respect to capacity, the capacity must first be determined over a range of different pH and flow rates.

1. Sample pH should be the same as that of the starting buffer. Filter the sample through a 0.22 μm or 0.45 μm filter to prolong the working life of the gel.
2. After the sample has been loaded, wash the gel with starting buffer until the base line is stable.

Elution

Red Sepharose CL-6B is a group specific adsorbent with affinity for a wide variety of biomolecules. Some proteins interact biospecifically with the dye due to its structural similarity to nucleotide cofactors, while others, such as carboxypeptidase G and interferon bind in a less specific manner by electrostatic and/or hydrophobic interactions with the aromatic anionic ligand.

- Specifically bound enzymes may be eluted by competitive elution with low concentrations of free cofactor, NAD^+ or $NADP^+$, in the buffer. Specifically bound biomolecules normally elute in the range 1-5 mM. In some cases up to 20 mM free cofactor has to be used. Either step or continuous gradients may be used.
- Less specifically bound biomolecules can be eluted with increased ionic strength. Elution is normally complete at salt concentrations of 3 M or less, NaCl or KCl. Either step or continuous gradients may be used.Elution can also be achieved by a change in pH or polarity (e.g. ethylene glycol, dioxan). Up to 50% ethylene glycol and up to 10% dioxan may be used.

Regeneration

Depending of the nature of the sample, Red Sepharose CL-6B may be regenerated for re-use by washing the gel with 2-3 bed volumes of alternating high pH (0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate + 0.5 M NaCl, pH 4.5) buffers. This cycle should be repeated 3 times followed by re-equilibration in binding buffer.

Cleaning

In some applications, substances like denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the column with 2 bed volumes of 6 M guanidine hydrochloride or 8 M Urea.

Alternatively, wash the column with 2 bed volumes of detergent in a basic or acidic solution. Use e.g. 0.1% non-ionic detergent, e.g. Triton X-100, in 1 M acetic acid. Remove residual detergent by washing with 5 bed volumes of 70% ethanol.

In both cases wash immediately with at least 5 bed volumes of binding buffer.

Storage

Freeze-dried Red Sepharose CL-6B should be stored below 8°C.

Swollen gel should be stored at 4-8°C in 0.1 M KH_2PO_4, pH 8.0 in the presence of a bacteriostat, i.e. 20% ethanol.

Ordering information

	Pack size	Code No.
Red Sepharose CL-6B	10 g	17-0528-01

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GE imagination at work