Macro-Prep®
Ion Exchange Media

Instruction Manual

BIO-RAD

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Section 1
Introduction

Macro-Prep ion exchange media are designed to provide high resolution and high capacity for preparative separations. The rigid methacrylate beads exhibit little shrinkage and swelling, making them suitable for both low- and medium-pressure chromatography. The macroporous media allow both small and large molecules to access exchange sites located throughout the chromatography bed. The physical structure of the media permits high flow rates at low backpressure.

Bio-Rad's chromatography media are manufactured in an ISO 9001 registered manufacturing facility. Manufacturing processes are audited and registered by National Quality Assurance Limited under the provisions of ANSI/ISO/ASQ 9001:2000. Bio-Rad Laboratories guarantees quality of product in unopened containers for 1 year from date of shipment. Regulatory support files are available upon request for Macro-Prep ion exchange chromatography media. If you need assistance validating the use of Macro-Prep media in a production process, contact your local Bio-Rad Process Chromatography representative.

Section 2
Intended Use

Macro-Prep ion exchange media are appropriate for capture, intermediate, and polishing purification steps. Due to their rigidity and unique surface chemistry, these media are particularly suited for flow-through operations requiring high throughput and high recovery of target molecules. The slightly hydrophobic base bead also provides selectivity for removal of trace contaminants such as residual host cell proteins, endotoxins, and viruses.

Macro-Prep media offer many advantages in both laboratory and process-scale applications. They are easy to use, allow a quick and reliable packing, and can be used for multiple cycles.

For maximum flexibility, the product offering includes Macro-Prep High Q strong anion exchange support, Macro-Prep DEAE weak anion exchange support, Macro-Prep High S strong cation exchange support, and Macro-Prep CM weak cation exchange support.

All Macro-Prep ion exchange media contain carboxyl groups. Depending on the media, pH conditions, and samples, the media can act in mixed mode. This property can be exploited to give unique selectivity, which may increase yield and purity.

Section 3
Media Screening

Macro-Prep ion exchange media are supplied prepacked in 1 ml and 5 ml cartridges for ease of screening. In addition, these media are part of the Media Sampler Pack, which includes Macro-Prep HIC, UNOsphere™ and CHT™ ceramic hydroxyapatite media. (See Ordering Information in Section 11 at the end of this instruction manual for a list of available items.) For the most up-to-date package options, visit our web site at www.bio-rad.com.

It is important with Macro-Prep ion exchange media to evaluate both conductivity and pH conditions. Since ion exchange media are often used after an affinity step, it may be necessary to adjust the salt concentration and/or the pH of the sample load to obtain optimal purification and recovery with Macro-Prep ion exchange media.

Section 4
Technical Specifications and Characteristics

Macro-Prep ion exchange media are shipped as a 50% slurry in 20% ethanol. Table 1 lists the properties of the Macro-Prep ion exchange media. The rigidity of these media allows high flow rates without bed compression (Figure 1). Testing in a small laboratory-scale column (10 mm ID X 100 mm length, 7.9 ml) showed no bed compression at a pressure of 7 bar and a flow rate of 40 ml/min (3,000 cm/hr). This property provides for high flow rates used during loading, column washing, equilibration, and cleaning.

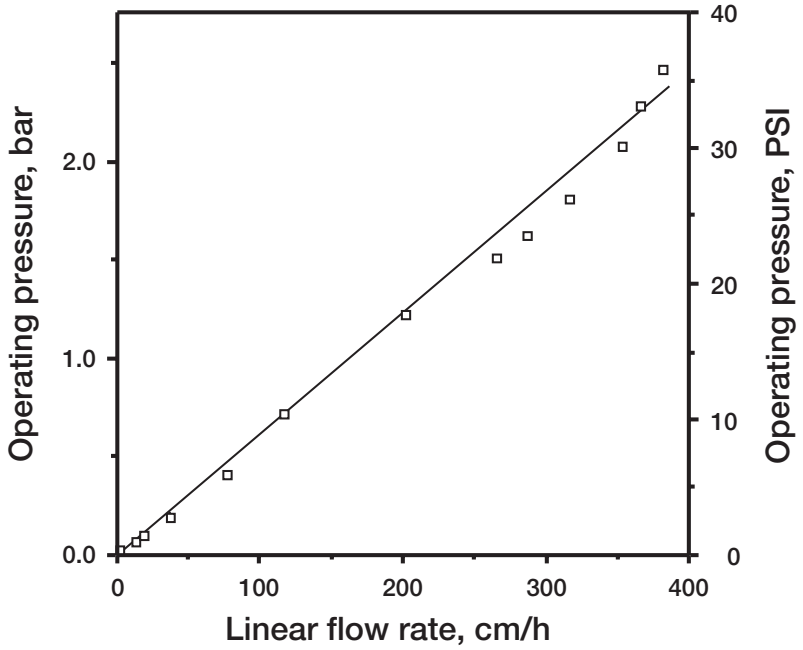

Fig. 1. Linear flow rate vs. operating pressure. Data for Macro-Prep High Q support was generated on a 14 cm (ID) X 17.4 cm (H) Amicon Moduline glass column with 1/4" plumbing. A Cole-Parmer Masterflex pump, size 18 neoprene tubing, Easy-Load pumphead was used. The eluent was water.

Table 1. Properties of Macro-Prep Ion Exchange Supports.

	Macro-Prep High Q Support	**Macro-Prep DEAE Support**	**Macro-Prep High S Support**	**Macro-Prep CM Support**
Type of ion exchanger	Strong anion	Weak anion	Strong cation	Weak cation
Functional ligand	$-N^+(CH_3)_3$	$-N^+(C_2H_5)_2$	$-SO_3^-$	$-COO^-$
Ionic capacity	400 ± 75 µeq/ml	175 ± 75 µeq/ml	160 ± 40 µeq/ml	210 ± 40 µeq/ml
Dynamic binding capacity	≥37 mg BSA/ml	≥30 mg BSA/ml	≥49 mg IgG*/ml	≥25 mg Hemoglobin/ml
Counterion	Cl^-	Cl^-	Na^+	Na^+
Nominal particle size†	50 µm	50 µm	50 µm	50 µm
Nominal pore size	1,000 Å	1,000 Å	1,000 Å	1,000 Å
Recommended maximum linear flow rate	3,000 cm/hr	3,000 cm/hr	3,000 cm/hr	3,000 cm/hr
Chemical stability				
1% SDS, 24 hr	Yes	Yes	Yes	Yes
6 M Guanidine-HCl, 24 hr	Yes	Yes	Yes	Yes
pH stability**	1–10	1–10	1–12	1–12
Volume changes				
pH 4–10	<1%	<1%	<3%	<1%
0.1–1.0 M NaCl	<5%	<5%	<9%	<4%
Autoclavable (121°C, 30 min)	Yes††	Yes††	Yes	Yes
Antimicrobial agent	20% ethanol	20% ethanol	20% ethanol	20% ethanol

* Human IgG

**Based on leachables, as determined by TOC (see Table 2)

† Based on underivatized beads

††Do not autoclave in OH^- form

Chemical Stability

Macro-Prep ion exchange media are stable in most aqueous buffer solutions commonly used in purification of biomolecules. These ion exchangers also withstand treatment in solutions of acid, detergents, chaotropic agents, and pH < 10, while retaining full functional performance. We do not recommend routine cleaning or operation of Macro-Prep High Q and DEAE media above pH 10. Macro-Prep ion exchange media should not be sanitized or stored in NaOH.

Table 2. Chemical Stability of Macro-Prep Ion Exchange Media: Total Organic Carbon (ppm) Leachables.

Exposure	High Q	DEAE	High S	CM
0.1 M HCl	0	0	4	0
0.2 M H_3PO_4	0	0	13	1
0.01 M NaOH	28	N/A	N/A	N/A
0.1 M NaOH	200	26	31	4
1.0 M NaOH	337	121	49	6

Each of the media (10 ml) was exposed to the specific reagent (40 ml) and mixed for 24 hr at 23°C. Extracts were analyzed on a Shimadzu total organic carbon analyzer, Model TOC 5000A.

Thermal Stability

Macro-Prep media can be autoclaved at 121°C for up to 30 min in deionized water as a slurry or a moist cake. Do not autoclave Macro-Prep High Q and DEAE media in OH^- form.

Section 5
Preparation for Use

Wash the ethanol storage solution from the medium with deionized water.

Small volumes of Macro-Prep media are easily washed in a Büchner funnel with 4–5 column volumes of water or a low ionic strength (<20 mM) buffer. For large volumes, it may be more convenient to pour the desired amount of medium into a suitable container, allow the medium to settle, and decant the ethanol solution. Add one column volume of deionized water, resuspend the medium, allow it to settle, and decant the supernatant. Repeat this procedure with buffer (approximately 4–5 times, or until the ethanol is removed), and then pack the column (see Column Packing). The medium may be prepared by decanting the excess ethanol solution and resuspending the medium in the application buffer prior to column packing (Column Packing, step 1).

Section 6
Column Packing

Columns can be packed in different ways depending on the type of column and equipment to be used. Macro-Prep ion exchange media can be packed using a combination of axial compression with constant pressure, constant flow, or suction-packing methods. To pack efficient columns, a 50% slurry is recommended. General guidelines for packing small and large columns are given below. Follow the instructions for the column hardware to be used. If you are unsure about a particular column, contact the column manufacturer or your local Bio-Rad Laboratories representative. Throughout this instruction, flow rate is expressed in centimeters per hour (cm/hr). The relationship between linear and volumetric flow rates as delivered by a pump is:

Linear flow rate (cm/hr) x Cross-sectional area of the column (cm^2) = Volumetric flow rate

Example: 300 cm/hr in a 14 cm diameter column = 300 cm/hr x πr^2 or 300 cm/hr x (3.14×7^2) cm^2 or 46,158 cm^3/hr or 769.3 ml/min.

Packing Small Columns

The following slurry packing method was designed to pack 25 ml of Macro-Prep media into 5–15 mm ID, low- to medium-pressure lab columns. Ideally, buffers are degassed prior to packing. Use a packing reservoir that holds ~100 ml.

1. Mix the prepared medium with an equal volume, 1:1 (v/v), of degassed packing buffer. For optimal column packing, use packing buffer containing 0.5–1.0 M salt with the same counterion as the start buffer.

   Caution: Do not mix with a magnetic stirbar, to avoid formation of small fines. Larger volumes of slurry may be mixed with an overhead stirrer at low to moderate speed.

2. Close the outlet of the column. Fill 10% of the column with degassed packing buffer. Remove any air bubbles that might be trapped in the bed support or column flow adaptor.

3. Add an appropriate amount of the media in a homogenous slurry to the column.

4. Fill the remainder of the column with packing buffer.

5. Connect the flow adaptor to the pump, fill it with buffer, and make sure it is free of air bubbles. Insert the flow adaptor to the column at a slight angle to avoid trapping air bubbles.

6. Open the column outlet and pump 4–5 column volumes through the column at a flow rate of about 100 cm/hr. Gradually increase the flow rate from 100 cm/hr to the flow rate at which the maximum allowable pressure for the

column is reached, or to the flow rate at which the maximum pressure for Macro-Prep is reached (7 bar or 102 psi). As the bed compresses with increasing flow rate, stop the flow and adjust the flow adaptor so it penetrates 1–2 mm into the media bed. Repeat the flow and adjustment cycle until the bed no longer compresses.

Note: Always pack the column at the highest flow rate your chromatography system permits, not exceeding the recommended maximum linear flow rate of 3,000 cm/hr. A well-packed bed will provide better resolution.

7. Attach the column to your chromatography system and purge with the starting buffer at the maximum flow rate to be used during operation. You should not see any further bed compression. If you do, adjust the flow adaptor 1–2 mm into the media bed.

8. Equilibrate the column in starting buffer. The column is now ready for sample application.

   Note: For optimal long-term use, do not exceed 80% of the maximum pressure attained during the column packing.

Packing Large Columns

In large columns, Macro-Prep media should be packed using a 50% slurry at constant pressure up to a maximum of 7 bar (102 psi). Pack with a constant flow rate that is approximately 25% greater than the anticipated maximum to be used during operation. Given the variety of industrial column hardware and packing skids, we recommend using standard operating procedures for packing provided by the column vendor. Bio-Rad EasyPack™ and GelTec™ columns are supplied with a movable piston. The GelTec™ column is packed by pumping the packing solution through the media bed at constant pressure or flow.

1. Packing begins with preparation of the tubing and the column. Clean and rinse the column well with packing buffer before packing. The rinse is also used to displace air from the column and its exit tubing. Make sure there is no air trapped in the bottom flow cell. After the air has been removed from the bottom flow cell and exit tubing, leave about 2 cm of liquid in the bottom of the column. Close the outlet valve(s).

2. In a suitable container, mix the packing buffer with the media to form a 50% slurry.

3. Pour or pump the slurry into the column.

4. Insert the movable piston and lower it to the surface of the liquid, making sure no air is trapped under the piston.

5. Pressurize the inflatable seal to 2 bar (29 psi), so that as the piston is lowered into the slurry, the buffer exits the top of the column through the inlet line. Be sure the inlet line is completely filled with packing buffer to avoid air being pumped back into the column.

6. Connect a pump and a pressure gauge, open all inlet and outlet valves, and start packing at constant flow rate or pressure. Keep the flow rate or pressure constant throughout the packing. Check the pressure at the column inlet. Do not exceed the pressure limit of column or medium.
7. Lower the piston as the bed compresses.
8. When the media bed no longer compresses, mark the bed height on the column tube, close the outlet valve, and stop the pump. The bed will start to expand in the column.
9. Lower the piston to within 1 cm of the surface of the media bed. Fully inflate the seal (4 bar), start the pump, open the valves, and continue packing.
10. Repeat steps 8 and 9 until there is a maximum of 1 cm between media bed surface and piston when the media bed is stable.
11. Close the bottom valve, stop the pump, disconnect the column inlet, and lower the piston to the gel bed surface. The column is ready for testing of column packing efficiency.

Section 7
Column Packing Evaluation

Packing efficiency should be tested before use and re-evaluated, as necessary. The values to be determined for the packed column are the height equivalent theoretical plate (HETP) or number of theoretical plates (N), and the asymmetry (A_s). The method involves applying a test sample of a low molecular weight substance that has no interaction with the media, e.g., 2 M NaCl. When using salt as the test substance, use a concentration of 2 M NaCl in water with 0.5 M NaCl in water as the elution buffer. Concentrated buffer solutions, e.g., 10X buffer concentrate, can also be used. HETP varies depending on the test conditions. Since HETP and A_s are used as reference values, it is important to keep conditions and equipment constant so results can be compared over time. Changes in solute, solvent, elution buffer, sample volume, flow rate, flow path, temperature, etc. influence results. For optimal results, ensure that the sample volume does not exceed 2.5% of the column volume, and maintain the flow rate between 75 and 150 cm/hr. The column HETP and/or calculated plate number, N, can be used as acceptance criteria for packed columns. If a UV absorbing substance is used as the test sample, use a UV absorbance monitor set at 280 nm. If 2 M NaCl is the sample, use a conductivity monitor; the elution buffer should be 500 mM NaCl. Apply the sample as close to the column inlet as possible to avoid dilution in the tubing and piping leading up to the column inlet. Calculate HETP, the number of theoretical plates (N), and A_s as follows:

$A_s = b/a$
$HETP = L/N$
$N = 5.54(V_e/W_{½h})^2$
L = Bed height (cm)
N = Number of theoretical plates

V_e = Peak elution volume or time
$W_{½h}$ = Peak width at peak's half height in volume or time
V_e and $W_{½h}$ should always be the same units

Peaks should be symmetrical. The best achievable asymmetry factor is 1. Values of 0.8 to 1.8 are excellent for large columns >30 cm in diameter. If functional performance is acceptable, A_s factors more distant from 1 put less demand on the column packing procedure in production. A change in the shape of the peak is usually the first indication of deteriorating performance. Peak asymmetry factor calculation:

$A_s = b/a$

a = Front section of peak width at 10% of peak height bisected by line denoting V_e.
b = Latter section of peak width at 10% of peak height bisected by line denoting V_e.

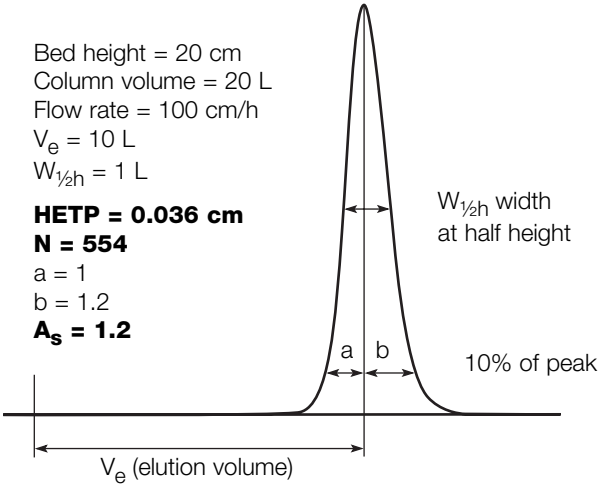

Fig. 2. A simulated chromatography profile from which HETP and A_s values are calculated.

Section 8
Operation and Maintenance

Macro-Prep ion exchange columns may be operated in two different modes. In bind-elute mode, the protein of interest (target molecule) binds to the medium during loading. It is subsequently eluted by a change of conductivity and/or pH. This allows removal of contaminants that do not bind to the medium and contaminants that may bind more weakly or more strongly than the protein of interest. In flow-through mode, the protein of interest does not bind during loading, while contaminants that may bind to the medium are removed. This approach can simplify chromatography conditions, conserve buffer, and increase recovery of the target molecule.

For flow-through mode, a linear flow rate of 300 cm/hr in a 20 cm bed is practical. If operating in a bind-elute mode, flow rates of 100–200 cm/hr are recommended. Lower flow rates may support higher capacity and better resolution.

All buffers commonly used for anion or cation exchange chromatography can be used with the appropriate ion exchange supports (see Table 3). The chemical stability and broad operating pH range of these ion exchangers allow the use of a variety of buffers. It is best to use buffering ions that have the same charge as the functional group on the ion exchanger, e.g., phosphate (–) with a cation exchanger, and Tris (+) with an anion exchanger. Buffers should be chosen so operating pH is within 0.5 pH unit from pK_a of buffer substance. The purification may be optimized by changing buffers, salts, pH, additives, and gradient.

Table 3. Common Buffers for Ion Exchange Chromatography.[1, 2, 3]

Type of Ion Exchanger	Buffer	Buffering Range
Cation	Acetic acid	4.8–5.2
	Citric acid	4.2–5.2
	HEPES	6.8–8.2
	Lactic acid	3.6–4.3
	MES	5.5–6.7
	MOPS	6.5–7.9
	Phosphate	6.7–7.6
	PIPES	6.1–7.5
	TES	6.8–8.2
	Tricine	7.8–8.9
Anion	Bicine	7.6–9.0
	Bis-Tris	5.8–7.2
	Diethanolamine	8.4–8.8
	Diethylamine	9.5–11.5
	L-Histidine	5.5–6.0
	Imidazole	6.6–7.1
	Pyridine	4.9–5.6
	Tricine	7.4–8.8
	Triethanolamine	7.3–8.3
	Tris	7.5–8.0

Note: Buffer solution of 50 mM is recommended.

Operating flow rates should not exceed 80% of the maximum pressure attained during the initial column packing in laboratory scale columns. If you do not have a pressure indicator, do not exceed flow rates in excess of 80% of the maximum flow rate used during packing. In process scale columns, do not exceed 70% of the maximum pressure attained during column packing.

Buffer Preparation

When preparing buffers for ion exchange chromatography, it is important that excess conductivity is not produced during buffer pH adjustment. For example, back-titration of Tris-HCl with NaOH elevates the conductivity, which will lower binding capacity. To prepare Tris buffer, begin with Tris base and titrate with Tris-HCl to the target pH. Apply the same principle with other buffers.

Column Equilibration

To equilibrate the column, wash with 1 to 2 column volumes (CV) of buffer containing 0.5–1.0 M of the buffer salt used in the start buffer. Follow with 3 CV of the start buffer or until pH and conductivity are stable.

Sample Preparation

Adjust salt concentration and pH as necessary to achieve desired selectivity for binding of target or contaminants. If sample conductivity exceeds conductivity of the column equilibration buffer, sample capacity may be reduced, or the target molecule may not bind. Adjustment of pH and conductivity of sample can be done by dilution or buffer exchange.

Sample Load and Adsorption

The sample load is determined empirically by loading and evaluating breakthrough of the molecule of interest. Sample volume is not a critical factor. Large volumes of dilute feed such as cell culture supernatant and clarified lysates may be loaded onto the medium without prior concentration.

Wash Through

When operating in a bind-elute mode, after loading of the sample onto the column, follow with 2 to 5 CV of the equilibration buffer. This will wash out unbound contaminants. In flow-through mode, after sample is completely through the column, regenerate the medium as described under "Regeneration" below.

Elution

Elute target molecules with a step or a linear gradient. The salt concentration at which the desired product elutes is predetermined with a linear gradient at small scale. With this knowledge, the pH and salt concentration are adjusted to eliminate the maximum amount of contamination before starting elution of the target.

Regeneration

After each run, the packed bed should be washed with 2–4 CV of a high salt buffer (0.5–2.0 M) to remove remaining bound material. It is important to do this before acid treatment to avoid precipitation of protein on the media. If the column no longer yields reproducible results, the medium may require more thorough cleaning and sanitization.

Cleaning-in-Place (CIP)

Acceptable CIP agents include 1% acetic acid/1% phosphoric acid with 0.4 M NaCl, up to 30% acetic acid, 1% Triton X-100, up to 70% ethanol or 30% isopropyl alcohol, 8 M urea and 6 M guanidine-HCl. Any of these agents can be combined in an appropriate cleaning protocol. As a general guide, we recommend the following:

1. Use high salt buffer for regeneration, as above.
2. For aggregated or precipitated proteins, or when dirty feedstock (e.g., crude lysate) had been used, wash with 3–5 CV of 6 M guanidine hydrochloride or 8 M urea at 100 cm/hr.
3. For lipids or hydrophobically bound contaminants, wash with 0.1% Triton X-100, or 20–70% ethanol or isopropyl alcohol, or 10–30% acetic acid. Use 3–5 CV at 100 cm/hr.
4. Remove additional contaminants with 0.4 M NaCl in 1% acetic acid/1% phosphoric acid (3–5 CV at 100 cm/hr).
5. If the column is to be used again immediately, wash with 2 CV of deionized water and 4–5 CV of starting buffer at 100 cm/hr. Check the conductivity and pH of the effluent to verify that the column is equilibrated in the starting buffer before loading the sample.

Sanitization and Storage

To sanitize and store between campaigns, wash the column with 3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5. Store the column at 4–40°C. When not in use, store the Macro-Prep ion exchange supports in either 1% acetic acid in 1% phosphoric acid (pH 1.5) or in 20% (v/v) ethanol solution or in 2% benzyl alcohol. The ion exchange supports may also be autoclaved at 121°C, 2 bar, in a neutral pH slurry, for up to 30 min and stored in one of the above solutions.

Shelf Life

Macro-Prep ion exchange supports are stable for at least one year in 20% ethanol when stored sealed in the original container at room temperature.

Section 9
Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative. In the USA, call 1-800-4BIORAD. For online product information, visit our web site at **www.bio-rad.com**

Section 10
References

1. Harris ELV and Angal S, Protein Purification Methods, A Practical Approach, IRL Press, Oxford (1989)
2. Scopes RK, Protein Purification, Principles and Practice, 2nd edn, Springer-Verlag, New York (1987)
3. Snyder LR and Kirkland JJ, Introduction to Modern Liquid Chromatography, 2nd edn, Wiley, New York (1979)

Section 11
Ordering Information

Catalog #	Description
732-0007	Econo-Pac® Macro-Prep DEAE Cartridge, 1 x 5 ml
732-0008	Econo-Pac Macro-Prep DEAE Cartridges, 5 x 5 ml
732-0009	Econo-Pac Macro-Prep DEAE Cartridges, 5 x 1 ml
158-0020	Macro-Prep DEAE Support, 25 ml
156-0020	Macro-Prep DEAE Support, 100 ml
156-0021	Macro-Prep DEAE Support, 500 ml
156-0022	Macro-Prep DEAE Support, 5 L
156-0023	Macro-Prep DEAE Support, 10 L
732-0001	Econo-Pac Macro-Prep CM Cartridge, 1 x 5 ml
732-0003	Econo-Pac Macro-Prep CM Cartridges, 5 x 1 ml
732-0005	Econo-Pac Macro-Prep CM Cartridges, 5 x 5 ml
158-0070	Macro-Prep CM Support, 25 ml
156-0070	Macro-Prep CM Support, 100 ml
156-0071	Macro-Prep CM Support, 500 ml
156-0072	Macro-Prep CM Support, 5 L
156-0073	Macro-Prep CM Support, 10 L
732-0026	Econo-Pac Macro-Prep High Q Cartridge, 1 x 5 ml
732-0027	Econo-Pac Macro-Prep High Q Cartridges, 5 x 5 ml
732-0028	Econo-Pac Macro-Prep High Q Cartridges, 5 x 1 ml
158-0040	Macro-Prep High Q Support, 25 ml
156-0040	Macro-Prep High Q Support, 100 ml
156-0041	Macro-Prep High Q Support, 500 ml
156-0042	Macro-Prep High Q Support, 5 L
156-0043	Macro-Prep High Q Support, 10 L
732-0066	Econo-Pac Macro-Prep High S Cartridge, 1 x 5 ml
732-0067	Econo-Pac Macro-Prep High S Cartridges, 5 x 5 ml
732-0068	Econo-Pac Macro-Prep High S Cartridges, 5 x 1 ml
158-0030	Macro-Prep High S Support, 25 ml
156-0030	Macro-Prep High S Support, 100 ml
156-0031	Macro-Prep High S Support, 500 ml
156-0032	Macro-Prep High S Support, 5 L
156-0033	Macro-Prep High S Support, 10 L
158-0100	Media Sampler Pack

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