IMAC, GST, Desalting, and Cartridge Cleaning Buffer Kits

Instruction Manual

Catalog Number

10017290

BIO-RAD

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Section 1
Introduction

1.1 Background

Polyhistidine and GST (glutathione-S-transferase) are the two most common affinity tags used for recombinant protein purification. To make purification of these tagged proteins fast and easy, Bio-Rad has developed optimized buffers and purification kits for IMAC (immobilized metal affinity chromatography, for His-tagged protein purification) and GST-tagged purification. For convenience, desalting and cartridge cleaning kits are also available.

The purification and cleaning kits come with concentrated buffers that can be diluted to 1x working solution by instruments such as the Bio-Rad Profinia™ Protein Purification System and Biologic DuoFlow™ and LP Chromatography Systems. Alternatively, the buffer can be manually diluted to 1x for use on gravity-flow, syringe, and spin-column formats.

1.2 Product Information

1.2.1 Product Configuration

Three basic kits are available for affinity purification and each is available with or without purification cartridges (see Table 1).

Table 1. Configuration for native IMAC, GST, and desalting and cartridge cleaning kits.

	Purification Kit (with Pre-Packed Bio-Scale™ Mini Cartridges)	Buffer Kit (with No Cartridges)
Native IMAC	620-0241 (1 ml) and 620-0242 (5 ml)	620-0239
GST	620-0243 (1 ml) and 620-0244 (5 ml)	620-0240
Desalting and cartridge cleaning	620-0228* (10 ml) and 620-0238* (50 ml)	620-0224

*Named as Profinia Desalting Purification Kits. They can be used for other purification instruments.

The Native IMAC and GST kits are sufficient for 10 purifications on 1 ml affinity cartridges (10 mg/ml binding capacity) or 2 purifications on 5 ml affinity cartridges.

Each buffer kit contains one set of buffers, while purification kits contain one or two sets of buffers depending on the cartridge size coming with the kits. Bio-Scale Mini IMAC, GST, or desalting cartridges contain:

- For 1 ml native IMAC and GST purification kits, 2 x 1 ml affinity cartridges and one set of buffers
- For 5 ml native IMAC and GST purification kits, 1 x 5 ml affinity cartridge and two sets of buffers
- For 10 ml desalting and cartridge cleaning purification kits, 2 x 10 ml desalting cartridge and one set of buffers
- For 50 ml desalting and cartridge cleaning purification kit, 1 x 50 ml desalting cartridge and two sets of buffers

The cartridge cleaning and storage buffers can be used for both affinity and desalting cartridges.

1.2.2 Buffer Components

Buffer components and concentrations are listed in Tables 2, 3, and 4.

Table 2. Formulations for buffers and solutions provided in the native IMAC kits.

Profinia Solution	Supplied As	Concentrated Formulation	Working 1x Formulation	Volume
Native IMAC lysis buffer	2x	600 mM KCl, 100 mM KH_2PO_4, 10 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 5 mM imidazole, pH 8.0	125 ml
Native IMAC wash buffer 1	2x	600 mM KCl, 100 mM KH_2PO_4, 10 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 5 mM imidazole, pH 8.0	125 ml
Native IMAC wash buffer 2	2x	600 mM KCl, 100 mM KH_2PO_4, 20 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 10 mM imidazole, pH 8.0	100 ml
Native IMAC elution buffer	2x	600 mM KCl, 100 mM KH_2PO_4, 500 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 250 mM imidazole, pH 8.0	100 ml

Table 3. Formulations for buffers and solutions provided in the GST Kits

Profinia Solution	Supplied As	Concentrated Formulation	Working 1x Formulation	Volume
GST lysis buffer	2x	300 mM NaCl, 20 mM Na_2HPO_4, 10 mM EDTA, pH 7.4	150 mM NaCl, 10 mM Na_2HPO_4, 5 mM EDTA, pH 7.4	125 ml
GST wash buffer	2x	300 mM NaCl, 20 mM Na_2HPO_4, 10 mM EDTA, pH 7.4	150 mM NaCl, 10 mM Na_2HPO_4, 5 mM EDTA, pH 7.4	200 ml
GST elution buffer	2x	40 mM glutathione, 200 mM Tris, 10 mM EDTA, pH 8.0	20 mM glutathione, 100 mM Tris, 5 mM EDTA, pH 8.0	100 ml

Table 4. Formulations for buffers and solutions provided in the desalting and cartridge cleaning kits.

Profinia Solution	Supplied As	Concentrated Formulation	Working 1x Formulation	Volume
Desalting buffer	5x	685 mM NaCl, 13.5 mM KCl, 21.5 mM Na_2HPO_4, 40.5 mM KH_2HPO_4, pH 7.0 (pH 7.4 upon dilution)	137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 8.1 mM KH_2PO_4, pH 7.4	200 ml
Cleaning solution 1	2x	1,000 mM NaCl, 100 mM Tris, pH 8.0	500 mM NaCl, 50 mM Tris, pH 8.0	125 ml
Cleaning solution 2	4x	2,000 mM NaCl, 400 mM NaOAc, pH 4.5	500 mM NaCl, 100 mM NaOAc, pH 4.5	125 ml
Storage solution	2x	4% $C_6H_5CH_2OH$ (benzyl alcohol)	2% $C_6H_5CH_2OH$ (benzyl alcohol)	200 ml

1.2.3 Product Compatibility

The buffers and Bio-Scale Mini cartridges have been tested and are compatible with competitors instruments and cartridges.

To use the pre-packed cartridges on chromatography systems other than the Profinia system, use the fittings/adapters listed in Table 5.

Table 5. Adapters/fittings for Bio-Scale Mini cartridges for instrument connection.

Instrument	Adaptors/Fittings
Biologic DuoFlow or LP	732-0113 (Luer ¼-28)
GE AKTA series	732-0111 (Luer-M6)

1.3 Storage Conditions

All unopened Profinia IMAC purification and buffer kits can be stored between 4 and 22°C. The GST kits can be stored at 22°C for up to two weeks. For long-term storage, the GST kits should be stored at 4°C. The labels on the outside of the kit box provide exact expiration dates.

Section 2
Purification Protocol

Different purification procedures can be used to purify proteins of interest depending on the application. Figure 1 is the flowchart for common recombinant protein purification. Below, two affinity purification procedures based on the instruments used are described.

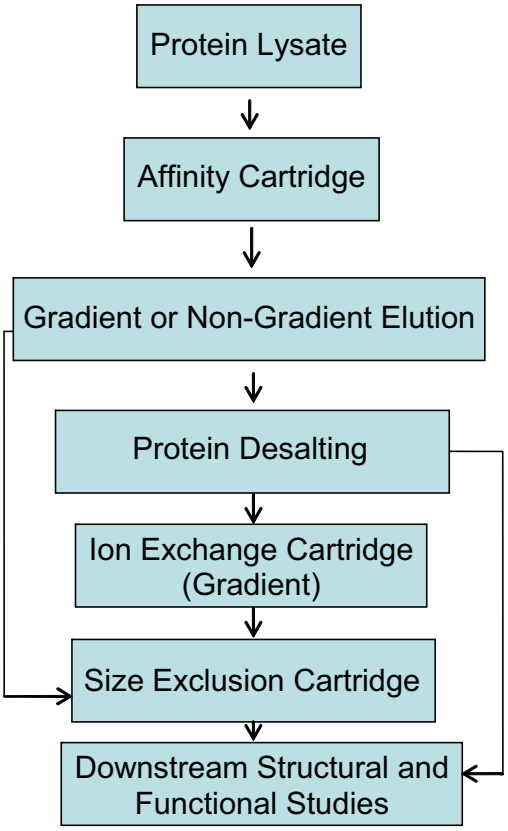

Fig. 1. Common recombinant protein purification flowchart starting from affinity purification.

Note: Native IMAC and GST kits can be used for affinity purification, while desalting and cartridge cleaning kits are used at the desalting step.

2.1 Run Purification on the Profinia System

Refer to Profinia Purification and Buffer Kits Instruction Manual (bulletin 10006044) Section 2 through 5.

2.2 Run Purification on Other Instruments

Purification method using 1 or 5 ml IMAC or GST purification cartridges.

1. Equilibrate the IMAC (GST) cartridge with DI water at 1 ml/min for 2 column volumes.
2. Equilibrate the IMAC (GST) cartridge with 1x IMAC (GST) wash buffer 1 at 1 ml/min for 5 column volumes.
3. Load the sample onto the IMAC (GST) cartridge at 1 ml/min.
4. Wash the IMAC cartridge with 1x IMAC wash buffer 1 at 1 ml/min for 6 column volumes.
5. Wash the IMAC cartridge with 1x IMAC wash buffer 2 at 1 ml/min for 6 column volumes. Note: (a) The UV trace should return to baseline after the wash. (b) GST kits contain only one wash buffer; omit this step for GST purification.
6. Elute the protein with 1x IMAC (GST) elution buffer at 1 ml/min for 4 or more column volumes, or until you see the UV trace return to baseline.
7. For cartridge cleaning and regeneration, the following procedure can be used with the Desalting and Cartridge Cleaning Buffer Kit.
    a. 1x cleaning solution 1 at 1 ml/min for 5 column volumes
    b. 1x cleaning solution 2 at 1 ml/min for 5 column volumes
    c. DI water at 1 ml/min for 5 column volumes
    d. 1x storage solution at 1 ml/min for 5 column volumes and store the cartridge

Note:

1. The suggested flow rate should be adjusted as needed depending on the binding properties of specific proteins and tag combinations.
2. For a 5 ml cartridge, the flow rate of 5 to 10 ml/min could be used.
3. This protocol could easily be adapted to purification done with gravity flow and spin columns.

Section 3
Use Native IMAC Kits for Denaturing Purification of His-Tagged Inclusion Bodies

Native IMAC kits are easy to adapt for use in denaturing IMAC purifications. His-tagged inclusion bodies can be solubilized in the presence of 6M guanidine hydrochloride or 6 to 8M urea and purified either as unfolded in presence of the denaturant or in the native form after on-column refolding of the protein in native buffer.

For denaturing IMAC, the following procedure can be used:

1. Add denaturant to wash buffers 1 and 2, and the elution buffer from the native IMAC buffer to the final concentration of 6M guanidine hydrochloride, or 6 to 8M urea, and add water to make the solution to 1x working buffer.
2. Purify the protein using the denaturing IMAC method preprogrammed on the Profinia protein purification system using the procedure for IMAC purification described in Section 2 of the Profinia Purification and Buffer Kits Instruction manual (bulletin 10006044).

To adapt on-column refolding of the purified protein, the following procedure can be followed:

Add denaturant to wash buffer 1 from Native IMAC buffer to the final concentration of 6M guanidine hydrochloride, or 6 to 8M urea, and add water to make the solution to 1x working buffer. Use a gradient wash from the high concentration of denaturant/low concentration of imidazole to no denaturant/low concentration of imidazole. Then elute the protein with a high concentration of immidazole.

Note: If step a gradient is used, wash buffer 1 should contain less concentrated denaturant, wash buffer 2 should contain no detergent, and the elution buffer should contain no denaturant but a high concentration of imdazole. This procedure can be done on the Profinia system.

However, on-column refolding/renaturing may not always work. Users may have to try the method to discover the optimal purification method for a particular protein.

Section 4
Sample Preparation

4.1 Preparing Lysates Prior to Purification

Lysates from *E. coli* cultures can be prepared using conventional sonication procedures with the lysis buffers supplied in each kit or by using compatible chemical lysis reagents. For *E. coli* cultures expressing medium to high levels of fusion proteins (≥10% of total protein), 200 ml of culture will normally yield sufficient material for a 1 ml cartridge purification, and 1,000 ml of culture will yield sufficient material for a 5 ml cartridge purification run. For cultures expressing protein at low levels (≤10% of total protein), the culture volumes will need to be determined empirically for each protein. Bacterial cultures can be grown in advance and centrifuged. The pellets can be stored at –70°C for several months and lysed at a convenient date for sample preparation.

4.11 Native Lysate Preparation (Native IMAC or GST Kits)

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of native IMAC lysis buffer or GST lysis buffer depending on the application (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate). Thoroughly resuspend the pellet by pipetting or vortexing.
3. As an optional step and to decrease viscosity, add a nuclease solution (DNase at 100 units/ml or Benzonase® at 25 units/ml).
4. Sonicate the lysate (on ice, using 25% output) four times at 1 min intervals.
5. Centrifuge at 16,000 x g for 20 min at 4°C to clarify the lysate.
6. Filter clarified lysate supernatant through a 0.45 µm filter to remove particulates.
7. Transfer the filtered lysate to a 15 ml or 50 ml sample tube for purification.
8. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. However, proteolysis or protein degradation can occur upon freezing and thawing, and the quality of the purified product may be compromised. This will have to be determined empirically for individual proteins. Upon thawing, refilter through a 0.45 µm filter, as precipitates often form after freezing.

4.12 Denaturing Lysate Preparation

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of denaturing IMAC lysis buffer containing urea (see Table 3) (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate). Thoroughly resuspend the pellet by pipetting or vortexing.
3. Sonicate the lysate (on ice, using 25% output) four times at 1 min intervals.
4. Centrifuge at 16,000 x g for 20 min at 4°C to clarify lysate.
5. Filter clarified lysate through a 0.45 µm filter to remove particulates. Transfer the filtered lysate to a 15 ml or 50 ml sample tube for purification.
6. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. See step 8 of Native Lysate Preparation for treatment upon freezing/thawing.

Native Lysate Preparation Using Bacterial Lysis/Extraction Reagent

(Recommended for IMAC procedures; binding capacities of GST fusion proteins will be decreased ~30% using chemical lysis methodologies.)

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of bacterial lysis/extraction reagent (Pierce Catalog #78243 or 78266) (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate). Thoroughly resuspend the pellet by pipetting or vortexing.
3. As an optional step and to decrease viscosity, add a nuclease solution (DNase at 100 units/ml or Benzonase at 25 units/ml) and incubate for 10 min at room temperature.
4. Centrifuge at 16,000 x g for 20 min to clarify the lysate.
5. Filter the clarified lysate through a 0.45 µm filter to remove particulates. Transfer the clarified lysate to a 15 ml or 50 ml sample tube for purification.
6. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. See step 8 of Native Lysate Preparation for treatment upon freezing/thawing.

4.14 Denaturing IMAC Lysate Preparation Using Bacterial Lysis/Extraction Reagent

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of bacterial lysis/extraction reagent (Pierce Catalog #78243 or 78266) (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate).
3. To decrease viscosity, add a nuclease (DNase at 100 units/ml or Benzonase at 25 units/ml) to the suspension and thoroughly resuspend by pipetting or vortexing. Let the solution incubate with gentle shaking 10 min at room temperature.
4. Centrifuge at 16,000 x g for 20 min at 4°C.
5. Discard the supernatant and add 10 volumes of Profinia denaturing IMAC lysis buffer containing urea (see Table 3) to the inclusion body pellet.
6. Vortex or mix well to thoroughly resuspend the pellet (~10 min).
7. Centrifuge at 16,000 x g for 20 min at 4°C to clarify lysate.
8. Filter the clarified lysate through a 0.45 µm filter to remove particulates. Transfer the clarified lysate to a 15 ml or 50 ml sample tube for purification.
9. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. See step 8 of Native Lysate Preparation for treatment upon freezing/thawing).

Section 5
Frequently Asked Questions and Troubleshooting

Proper Storage of Solutions and Kits

- All of the kits can be stored at 22°C (room temperature) for short periods of time, either upon receipt or during normal usage. For the kits that have labile reagents (glutathione in GST kits), 4°C is the recommended long-term storage temperature
- Once opened and used with the instrument, all reagents should be stored at 4°C for up to 3 months. After insertion into the instrument, the solutions are no longer sterile and require 4°C storage
- Once opened and used with the instrument, check the solutions for particulates and clarity before reusing. If there is any indication of contamination or microbial growth, the solution should discarded

Addition of Additives or Reagents to the Solutions

- The solutions are provided as concentrates and are diluted by the Profinia instrument. If it is desired to add an additional component to one of the solutions (that is, a protease inhibitor), it should be added as a concentrate and brought to the final concentration of the solution (that is, final 2x concentration for the affinity buffers). The volume to be added should be minimized so that it does not dramatically alter the final concentration of the solution labeled on the bottle

Addition of Urea and Glutathione

- Urea (not provided) and glutathione should be added to the buffers on the day purification starts. Any unused solution can be stored at 4°C for up to 7 days
- For longer-term storage, any unused buffer can be stored at –20°C and used within a 3-month period. Upon thawing, check for any particulates and if necessary, heat in a 37°C waterbath to dissolve precipitates. Always refilter solutions through a 0.2 µm filter if particulates are present. Discard if microbial growth is evident

Leftover Buffer After 10 Runs

- Each kit contains sufficient reagents for 10 purification runs (equivalent to 10 ml of resin). Depending upon which method is used (standard vs. extended wash, rerun of same method, insertion of new cartridge vs. using the same cartridge, etc.), some bottles may not be completely depleted, while others may be nearly empty. To minimize the risk of contamination, it is recommended to discard any unused buffers after 10 runs rather than pool multiple old lots of buffer to refill bottles

Viscous Lysates

- The lysis buffers supplied in each kit are optimized for lysis by sonication. A ratio of 10 parts 1x buffer to 1 part cell pellet (v/w) is ideal for lysing cell pellets. Depending upon the density of the culture, the power of the sonicator, and the condition of the sonication tip, the final viscosity of the lysate may vary. Extremely viscous lysates create system backpressure and run the risk of slowing down the flow during sample loads
- To decrease viscosity, a nuclease should be added prior to sonication. Benzonase Merck KGaA Corp. or DNase are commonly used nucleases

Large Sample Volumes

- When low protein expression requires large culture volumes (>2 L starting culture), the lysate loading volume can exceed 45 ml, which exceeds the volume that can be used in a standard sample tube. In these instances, bacterial pellets can be lysed at 5:1 (v/w) ratios. When concentrating starting samples at 5:1 ratios, it is **strongly recommended** to add a nuclease to minimize sample viscosity

Section 6
Legal Notices

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under U.S. patent 5,284,933 and U.S. patent 5,310,663, including foreign patents (assignee Hoffman-LaRoche).

Expression and purification of GST fusion proteins may require a license under U.S. patent 5,654,176 (assignee Chemicon International).

Benzonase is a trademark of Merck KGaA Corp.

Section 7
Ordering Information

Catalog #	Description
Basic Affinity Purification/Buffer Kits	
620-0239	**Native IMAC Buffer Kit**, includes IMAC purification buffers
620-0241	**Native IMAC Purication Kit**, 1 ml, includes one set of IMAC purification buffers and 2 x 1 ml IMAC cartridge
620-0242	**Native IMAC Purification Kit**, 5 ml, includes two sets of IMAC purification buffers and 1 x 5 ml IMAC cartridge
620-0240	**GST Buffer Kit**, includes GST purification buffers
620-0243	**GST Purification Kit**, 1 ml, includes one set of GST purification buffers and 2 x 1 ml GST cartridges
620-0244	**GST Purification Kit**, 5 ml, includes two sets of GST purification buffers and 1 x 5 ml GST cartridge
620-0224	**Desalting and Cartridge Cleaning Buffer Kit**, includes desalting and column cleaning and storage buffers

Catalog #	Description
620-0228	**Profinia Desalting Purification Kit**, 10 ml, includes one set of desalting and cartridge buffers and 2 x 10 ml desalting cartridges
620-0238	**Profinia Desalting Purification Kit**, 50 ml, includes two sets of desalting and cartridge buffers and 1 x 50 ml desalting cartridge

Profinia Purification Kits

620-0225	**Profinia Native IMAC Purification Kit**, 1 ml, includes Profinia native IMAC buffer kit, 2 x 1 ml IMAC and 2 x 10 ml desalting cartridges
620-0235	**Profinia Native IMAC Purification Kit**, 5 ml, includes 2 Profinia native IMAC buffer kits, 1 x 5 ml IMAC and 1 x 50 ml desalting cartridge
620-0226	**Profinia GST Purification Kit**, 1 ml, includes Profinia GST buffer kit, 2 x 1 ml GST and 2 x 10 ml desalting cartridges
620-0236	**Profinia GST Purification Kit**, 5 ml, includes 2 Profinia GST buffer kits, 1 x 5 ml GST and 1 x 50 ml desalting cartridge
620-0228	**Profinia Desalting Purification Kit**, 10 ml, includes desalting and cartridge cleaning buffer kit, 2 x 10 ml desalting cartridges
620-0238	**Profinia Desalting Purification Kit**, 50 ml, includes 2 desalting and cartridge cleaning buffer kits, 1 x 50 ml desalting cartridge

Profinia Buffer Kits

620-0221	**Profinia Native IMAC Buffer Kit**, includes purification buffers, cleaning and storage solutions; sufficient for 10 applications for 1 ml cartridge
620-0223	**Profinia GST Buffer Kit**, includes purification buffers, cleaning and storage solutions, glutathione reagent; sufficient for 10 applications for 1 ml cartridge
620-0224	**Desalting and Cartridge Cleaning Buffer Kit**, includes desalting buffer, cleaning and storage solutions; sufficient for 10 applications for 10 ml cartridge

Profinia Starter Kits

620-0229	**Profinia Native IMAC Starter Kit**, includes Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, *E. coli* lysate
620-0230	**Profinia GST Starter Kit**, includes Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, *E. coli* lysate

Catalog #	Description

Profinia Reagents

620-0203	**His Antibody**, 100 µl, 1 mg/ml
620-0204	**GST Antibody**, 100 µl, 1 mg/ml
620-0233	**His and GST Purification *E. coli* Control Lysate**, lyophilized
620-0202	**Glutathione Pack**, 1.23 g

Bio-Scale Mini™ Affinity and Desalting Cartridges

732-4610	**Bio-Scale Mini Profinity IMAC Cartridges**, 5 x 1 ml
732-4612	**Bio-Scale Mini Profinity IMAC Cartridge**, 1 x 5 ml
732-4614	**Bio-Scale Mini Profinity IMAC Cartridges**, 5 x 5 ml
732-4620	**Bio-Scale Mini Profinity GST Cartridges**, 5 x 1 ml
732-4622	**Bio-Scale Mini Profinity GST Cartridge**, 1 x 5 ml
732-4624	**Bio-Scale Mini Profinity GST Cartridges**, 5 x 5 ml
732-5304	**Bio-Scale Mini Bio-Gel™ P-6 Desalting Cartridges**, 5 x 10 ml
732-5312	**Bio-Scale Mini Bio-Gel P-6 Desalting Cartridge**, 1 x 50 ml
732-5314	**Bio-Scale Mini Bio-Gel P-6 Desalting Cartridges**, 5 x 50 ml
732-4600	**Bio-Scale Mini Aff-Prep Protein A Cartridges**, 5 x 1 ml
732-4602	**Bio-Scale Mini Affi-Prep Protein A Cartridge**, 1 x 5 ml
732-4200	**Bio-Scale Mini UNOsphere SUPrA™ Cartridge**, 1 x 1 ml
732-4201	**Bio-Scale Mini UNOsphere SUPrA Cartridges**, 5 x 1 ml
732-4202	**Bio-Scale Mini UNOsphere SUPrA Cartridge**, 1 x 5 ml
732-4646	**Bio-Scale Mini Profinity eXact™ Cartridges**, 2 x 1 ml
732-4647	**Bio-Scale Mini Profinity eXact Cartridges**, 4 x 1 ml
732-4648	**Bio-Scale Mini Profinity eXact Cartridge**, 1 x 5 ml

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