



MULTI-PREP® Columns and 96 Well Deep-Well Plates for Sample Preparation

Instruction Manual

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**Biochemical
Diagnostics, Inc.**

A Kova International Company

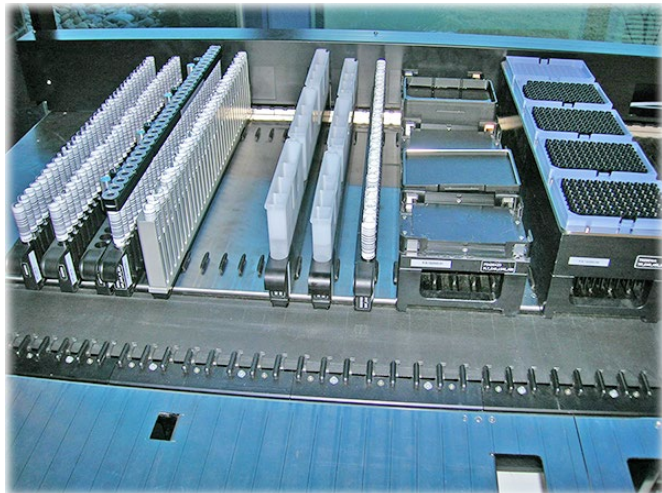
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Section 1

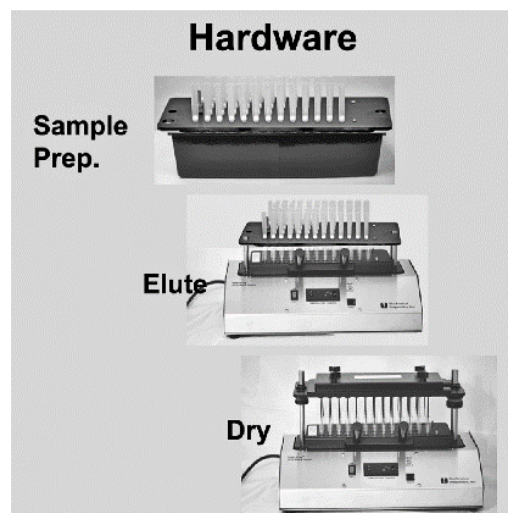
Introduction

Prefilled MULTI-PREP® columns are polypropylene columns containing non-ionic and ion exchange resins. These columns combine the analytical quality and wide selection of Biochemical Diagnostics resins with the convenience of disposable columns and are especially useful in sample preparation for GC/MS and LC/MS analysis. Whether its automation or manual sample preparation we have you covered.

ROBOTICS



MANUAL PREPARATION



Section 2

Column Descriptions

GC/MS Grade Extraction Columns:

Two different column configurations are currently available, each with the same bed configuration but with different size reservoirs,

15 mL and 3 mL. The 96 deep-well plates are also available with the same beds as are available in the individual columns. This feature enables the operator to interchange between manual and automated methods without varying sample or reagent volumes. The 15 mL reservoir column is available in both amber color to help protect light sensitive compounds, and natural opaque white and may be used manually or with robotic systems. The smaller 3 mL syringe barrel columns and the 96 deep-well plates are compatible with all robotic systems.

A variety of packings are available in these columns including non-ionic polymers, mixed bed resins, and ion exchangers and several silica gel based bonded phases.

The MULTI-PREP® GV-65 is a rugged gravity flow column, packed with a 100-200 mesh particle size modified copolymer bead, and is of the hydrophobic non-ionic type. GV-65 is available in a 15 mL reservoir column which is amber to protect compounds sensitive to light, and in two different styles of three mL syringe barrel style columns ideal for manual or robotic applications.

The MULTI-PREP® GV-65C column is a 100-200 mesh particle size gravity flow mixed mode type exhibiting both hydrophobic-non-ionic and cation exchange characteristics. The GV-65C is available in an opaque white column to distinguish it from the GV-65 amber column and in 3 mL syringe barrel columns. The bed volume is identical in both column sizes and may be used interchangeably.

LC/MS Grade Extraction Columns:

The MULTI-PREP® Ultra series of deep-well plates is unique in design and function:

The incorporation of a 0.45 or 0.22 micron membrane sandwiched between two 8 micron square filters exposes a large surface area which allows solutions to flow easily.

We manufacture the new plates in several ways:

A. Without SPE resin - Ultra hydrophilic 0.45 or 0.22 micron filter membrane.

Application:

1. Filtering elution solvent from a separate sample preparation.
2. Dilute and shoot up to 500 microliters of urine or oral fluid.

B. With SPE resin

Application:

A second configuration incorporates a small bed of resin, using either the cation exchange mixed bed (GV-35C), or the strong anion exchange resin (GVSA) for sample cleanup and elution all in one. The resin bed capacity is optimized for a sample size of 500 microliters or less.

Section 3

Instructions for Use

A typical non-ionic broad screen SPE method using the GV-65 mixed bed resin:

SPE column/96 well plate conditioning – All liquids flow by gravity

(please see extraction methods for individual drug extraction details)

1. Activate GV-35C resin with 1 mL of Methanol. Wait 2 minutes.
2. Wash GV-35C resin twice with 200 µL of 0.25M Phosphate Buffer, pH 9.1.
3. Proceed to Sample Extraction within 20 min. of column conditioning.

Sample extraction – All liquids flow by gravity

1. Transfer samples onto preconditioned columns or 96 well plate.
2. Wash with 0.5 mL 0.25M Phosphate Buffer, pH 9.1.
3. Dry the resin by applying positive pressure or, vacuum adjusted to at least 7" Hg for 3-5 minutes.

Sample elution – All liquids flow by gravity

1. Elute into a borosilicate glass tube or 96 well collection plate with 400 µL of Ethyl Acetate:Isopropanol (85:15) and allow to flow through by gravity.
 2. Add an additional 400 µL of Ethyl Acetate:Isopropanol (85:15) and allow to flow through by gravity.
 3. Following elution the eluate must be completely dried down under Nitrogen or Argon and reconstituted with a diluted solution of methanol compatible with the mobile phase used for LC/MS analysis.
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Section 4

Exchange Characteristics

Anion Exchange Resins

MULTI-PREP® GVSA Anion Exchange Resins are strong (type 1) ion exchange resins. They use a trimethylbenzylammonium group as the exchange site. They are supplied in the chloride form (with a chloride ion occupying the exchange site). This form is very stable and has an excellent shelf life.

Anion Exchange Resin Characteristics

Type: strong base anion exchanger

Active Group: trimethylbenzylammonium

% divinylbenzene: 2%, 4%, or 8%

Supplied Ionic Form: Cl⁻

Moisture Content: 43% to 48%

Volume Change: Cl⁻ to OH⁻ is +20%

pH Range: 0 to 14

Selectivity: Cl⁻/OH⁻ is about 25

Order of Selectivity: I⁻ > NO₃⁻ > Br⁻ > Cl⁻ > acetate > OH⁻ > F⁻

Cation Exchange Resins

MULTI-PREP® GV-35C/65C Cation Exchange Resins are of a mixed bed type containing both non-ionic moieties and strong acid (type 50) ion exchange resins. They contain a sulfonic acid active group as the exchange site. They are supplied with a hydrogen ion occupying the exchange site.

Cation Exchange Resin Characteristics

Type: strong acid cation exchanger

Active Group: sulfonic acid

Supplied Ionic Form: H⁺

pH Range: 0 to 14

Order of Selectivity: Ba⁺⁺ > Rb⁺⁺ > Ca⁺⁺ > Mg⁺⁺ > Be⁺⁺ > Ag⁺ > Cs⁺ > Rb⁺ > K⁺ > NH₄⁺ > Na⁺ > H⁺ > Li⁺

Section 5

Flow Rate Characteristics

The flow rate of an ion exchange resin is primarily determined by the particle size of the resin. Using gravity flow, the expected flow rate for 100-200 mesh resin (74-149 μ) in prefilled MULTI-PREP® columns is approximately 1-2 ml/minute. The flow rate for 200-400 mesh resin (37-74 μ) is approximately 4-6 mL/minute.

The MULTI-PREP® Ultra columns and plates with sub-micron filters have a slower flow rate than the GC/MS grade resins and flow at approximately 0.5 mL/minute. Positive pressure or vacuum assist may be used to induce faster flow rates but should not exceed 1-2 mL/min.

Section 6

Temperature Limits

Increases in temperature tend to decrease resin selectivity, and can be used to decrease elution times. Increased temperature also increases exchange kinetics, resulting in sharper resolution. However, some temperature limits must be observed with ion exchange resins.

Table 3

Maximum temperature for each resin packing.

Resin Packing	Temperature (°C)
GV-65 resin	80
GV-65C resin	80
GVSA resin	80

Table 2 gives the chemical compatibility of prefilled columns. While the column is very resistant, the compatibility of the resin must be considered.

Table 2.

Chemical Compatibility of column

Chemical	Compatibility*
Acetic acid, 50%	S
Acetone	S
Acetonitrile	S
Ammonium hydroxide	S
Chloroacetic acid	S
Chromic acid, 50%	U
Diethyl ether	L
Dimethyl formamide	S
Dimethylsulfoxide	S
Ethyl alcohol	S
Ethylene glycol	S
Formamide	S
Glacial acetic acid	S
Glycerine	S
Hydrochloric acid, 35%	S
Isobutyl alcohol	S
Isopropyl alcohol	S
Methanol	S
Perchloric acid	U
Phosphoric acid	S
Propylene glycol	S
Sodium hydroxide, 50% to Sat.	L (U for anions)
Sulfuric acid, 60%	S (U for anions)
Sulfuric acid, 98%	U
Urea, 8	M
S	

* S = Satisfactory U = Unsatisfactory L = Low

Section 7

Chemical Troubleshooting

Section 8

Compatibility

1. Variation in color (lot-to-lot): Sometimes the color of the resin may vary from lot to lot. This will not affect the ion exchange capacity of the resin.
 2. Unusual odor: Amines will sometimes cleave from anion exchange resin after pro- longed storage. This is not unusual. They can be removed easily by rinsing with 2-3 bed volumes of methanol.
 3. Effluent is red: After prolonged storage, sulfonic acid will sometimes leach from the strong cation exchange resin, yielding a red effluent. Wash the resin with water until the color is no longer visible.
 4. The GV-65, 96 Deep-well Microtiter plates, and GVSA columns are designed to flow freely by gravity. If liquids do not flow freely there is probably air trapped within the column bed or frits. Tapping the column mounting plate onto the vacuum box should initiate flow. Precipitates or sediment poured onto the column can also hinder or block the flow of liquids through the column.
- If you have any questions about using pre- filled columns, call our toll-free technical services number, 1-800-223-4835 (in the U.S.), or contact your local representative.

Section 9
Ordering Information

<u>Catalog #</u>	<u>Product Description</u>
14100620	MULTI-PREP® GV-65 Columns, non-ionic, <i>amber color</i> , 15 mL reservoir, Pkg. of 100
14100820	MULTI-PREP® GV-65 Columns, non-ionic, 3 mL Syringe Barrel, Pkg. of 100
14101660	MULTI-PREP® GV-65 96 Deep Well Microtiter plate, non-ionic, pkg. 5
14300910	MULTI-PREP® GV-65C Columns, non-ionic/Cation Exchange, <i>Natural</i> , 15 mL reservoir, pkg. 100
14100920	MULTI-PREP® GV-65C Columns, non-ionic/Cation Exchange, 3 mL Syringe, pkg. 100
14201360	MULTI-PREP® GV-65C Deep Well Microtiter Plate, pkg. 5
14400041	MULTI-PREP® Ultra-35C 96 Deep Well Microtiter Plate, with 0.45 micron hydrophilic filter pkg. 5
14200820	MULTI-PREP® GVSA-200 Columns, Amber, 15 mL reservoir, pkg. 100
14101920	MULTI-PREP® GVSA-200 Columns, 3 mL Syringe Barrel, pkg. 100

Filter Plates (without resin)

- 14400033 MULTI-PREP® Ultra 96 Deep Well Microtiter Plate, with 0.45 micron hydrophilic filter, pkg.5
14400031 MULTI-PREP® Ultra 96 Deep Well Microtiter Plate, with 0.25 micron hydrophilic filter, pkg.5

Note: Special order prefilled are available with the chromatographic material of your choice. A minimum order of 1,000 columns is required for special order. Prices quoted on request.

Accessories

<u>Catalog #</u>	<u>Product Description</u>
14024001	MULTI-PREP® Microplate flow inducer
14024000	MULTI-PREP® Crossing Guard™ for 96 well plate
14020001	MULTI-PREP® 28 place workstation
14058048	MULTI-PREP® SP/E 48 place workstation

Please note the DETECTABUSE brand has been changed to MULTI-PREP on all extraction products.



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