



extractman®

User's Guide



 **GILSON®**

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LT310210-01

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Safety

Read this section before operating the instrument. For safe and correct use of the instrument, operating and service personnel must follow all instructions contained in this guide when installing, cleaning, and maintaining the instrument.

Failure to comply with these precautions or with warnings described in the user's guide violates safety standards of design, manufacture, and intended use of the instrument. Gilson assumes no liability for customers failing to comply with these requirements.

Safety Notices

The following safety notices may appear in this document:

 WARNING	<p>WARNING indicates a potentially hazardous situation which, if not avoided, may result in serious injury.</p>
 CAUTION	<p>CAUTION indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury.</p>
NOTICE	<p>NOTICE indicates a potentially hazardous situation which, if not avoided, may result in equipment damage.</p>

Liquids

Observe safe laboratory practices when handling liquids. If working with biological samples or chemical substances, ensure that there is proper ventilation, and wear personal protective equipment (PPE), such as safety glasses, gloves, and protective clothing at all times.

Refer to the Material Safety Data Sheets for solvents before use.

Magnets

This instrument contains a strong magnet, which may affect devices or media sensitive to magnetism.

Sécurité

Merci de lire attentivement cette section avant toute installation ou utilisation de l'instrument. Pour une utilisation correcte et en toute sécurité de l'instrument, il est nécessaire que le personnel qui utilise et réalise la maintenance de l'instrument, suive les instructions contenues dans ce guide lors de l'installation, du nettoyage et de la maintenance de l'instrument.

Le non-respect de ces précautions ou des avertissements spécifiques mentionnés dans ce guide compromet les normes de sécurité de conception, de fabrication et d'utilisation prévue de l'instrument. Gilson décline toute responsabilité en cas d'incapacité du client à se conformer à ces exigences.

Notes de Sécurité

Les notes de sécurité suivantes peuvent apparaître dans ce document:

 WARNING	<p>WARNING (AVERTISSEMENT) indique une situation potentiellement dangereuse qui, si elle n'est pas évitée, peut entraîner des blessures graves.</p>
 CAUTION	<p>CAUTION (ATTENTION) indique une situation potentiellement dangereuse qui, si elle n'est pas évitée, peut entraîner des blessures mineures ou légères.</p>
NOTICE	<p>NOTICE (AVIS) indique une situation potentiellement dangereuse qui, si elle n'est pas évitée, peut entraîner des dommages matériels.</p>

Liquides

Respectez les pratiques de sécurité du laboratoire lors de la manipulation de liquides. Si vous travaillez avec des échantillons biologiques ou des substances chimiques, assurez-vous que la ventilation est adéquate et portez en permanence un équipement de protection individuelle (EPI), tel que: lunettes, gants et vêtements de protection.

Reportez-vous aux Fiches de Données de Sécurité pour les solvants avant toute utilisation.

Magnets

Cet instrument contient un aimant puissant susceptible d'affecter les éléments sensibles au magnétisme.

Description

EXTRACTMAN® leverages proprietary Exclusion-based Sample Preparation (ESP™) technology to gently and quickly target proteins from a variety of samples, including weakly-bound protein complexes that traditional isolation techniques leave behind. The sliding handle processes up to four samples simultaneously in as few as 30 seconds.

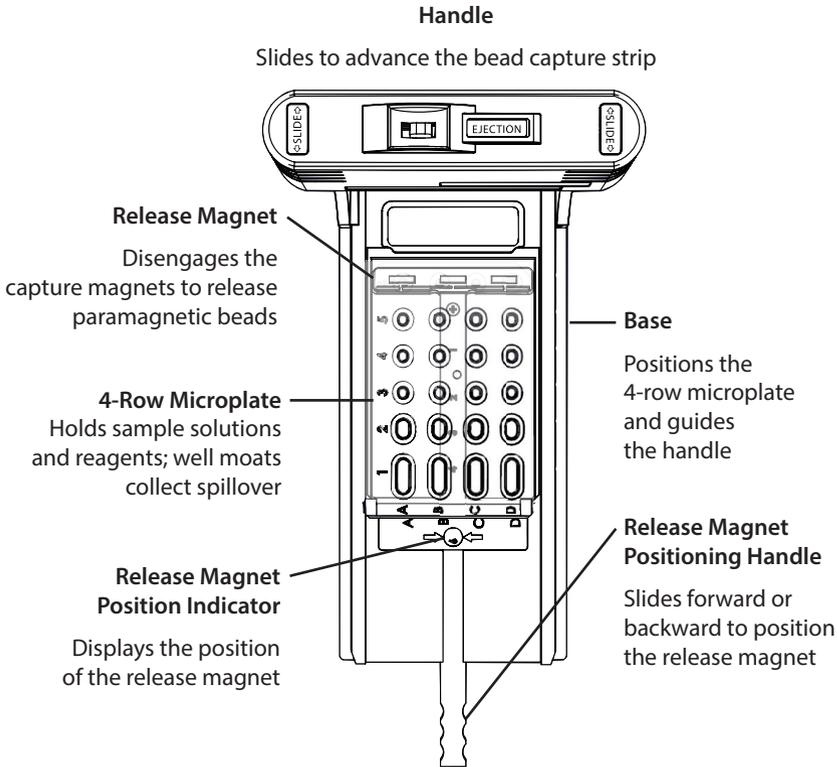


Figure 1: EXTRACTMAN® Component Descriptions

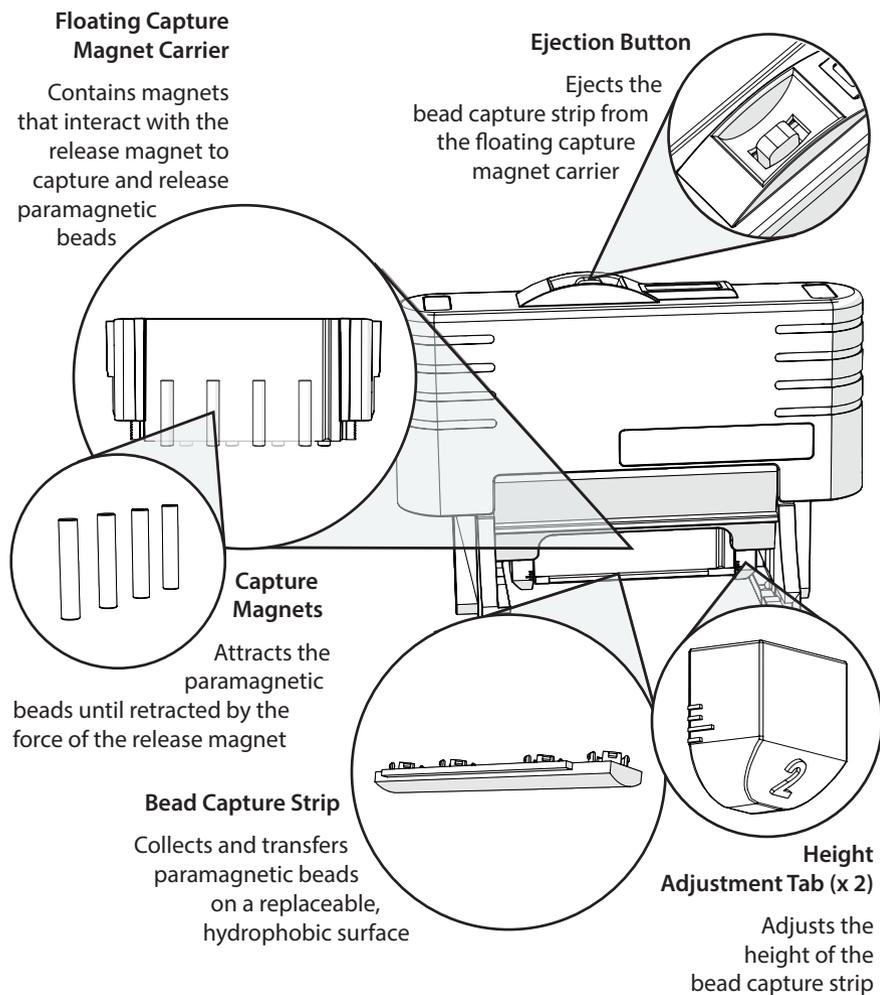


Figure 2: EXTRACTMAN® Handle Description

Unpack

EXTRACTMAN® is delivered with most major components already assembled. Keep the original container and packing assembly in case the instrument must be returned to the factory.

Unpack

1. Open the cardboard box.
2. Remove the documentation.
3. Remove the foam insert.
4. Remove EXTRACTMAN® and accessories.
5. Remove the plastic sleeves in the floating capture magnets carrier.

Standard Equipment

The following items are considered standard equipment and are provided with EXTRACTMAN®:

- Bead capture strip rack
- Bead capture strips (x 5)
- 4-row microplates (x 5)

Documentation

The following documents are included with EXTRACTMAN®.

- EXTRACTMAN® User's Guide
- EXTRACTMAN® Quick Start Guide
- Declaration of Conformity

Accessories

Required

Some accessories are required, but are ordered separately:

- Paramagnetic beads
- Pipette
- Tubes

Optional

- Vortex mixer

Repack

If it is necessary to repack and return EXTRACTMAN®, follow the instructions below. Please refer to [Repair and Return Policies](#) on page 42 before returning EXTRACTMAN®.

1. Place the bottom foam insert with the cavities facing up in the bottom of the original cardboard box.
2. Set the base on the long edge in the slot shown.
3. Set the handle in the slot shown below with the EXTRACTMAN® logo facing up.
4. Replace the top foam insert. Follow the instructions found in [Return Procedure](#) on page 43.

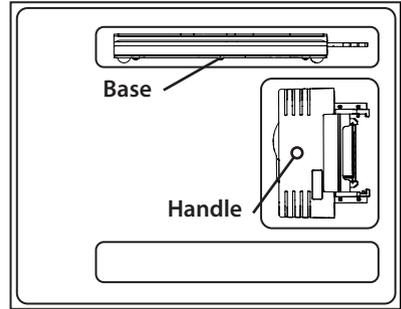


Figure 3: Repack EXTRACTMAN®

Gilson, Inc. and its worldwide network of representatives provide customers with the following types of assistance: sales, technical support, applications, and instrument repair.

If you need assistance, please contact your local Gilson representative. Detailed contact information can be found at www.gilson.com.

Trademarks

The following trademarks may appear in this document.

- Agencourt DNAdvance™ is a trademark of Beckman Coulter, Inc.
- Dynabeads® is a registered trademark of Thermo Fisher Scientific, Inc.
- Exclusion-based Sample Preparation (ESP™) is a trademark of Salus Discovery, LLC.
- MagneSil® is a registered trademark of the Promega Corporation.
- Qubit® is a registered trademark of Thermo Fisher Scientific, Inc.
- Triton™ is a trademark of the Dow Chemical Company or an affiliated company of Dow.
- Tween® is a registered trademark of Croda, Inc.

All other trademarks within are trademarks or registered trademarks of Gilson, Inc.

Technical Specifications

EXTRACTMAN®

<i>Technical Specification</i>	<i>Definition</i>	
Dimensions (W x D x H)	Fully Extended: 4.5 x 16.5 x 5.5 in. (11.5 x 42 x 14 cm) Fully Retracted: 4.5 x 12.5 x 5.5 in. (11.5 x 32 x 14 cm)	
Environmental Conditions	Indoor Use Minimum Temperature: 4°C (39°F) Maximum Temperature: 83°C (180°F)	
Liquid Contact Materials	<i>Description</i>	<i>Materials</i>
	4-Row Microplate	Polypropylene
	Bead Capture Strip	
Sterilization Techniques	<i>Component</i>	<i>Technique</i>
	Handle	Wipe down with a solution of 10% ethanol or 10% bleach.
	Base	
	Release Magnet Positioning Handle	
	4-Row Microplate	Autoclave for pre-use sterilization. Gilson recommends discarding 4-row microplates and bead capture strips after each use.
	Bead Capture Strip	
Weight	4.1 lbs (1.9 kg)	

Base

1. Position the base of EXTRACTMAN® on a level surface.
2. Slide the release magnet positioning handle to Well Column 2 (shown in the release magnet position indicator) or to the well column immediately following the input well.

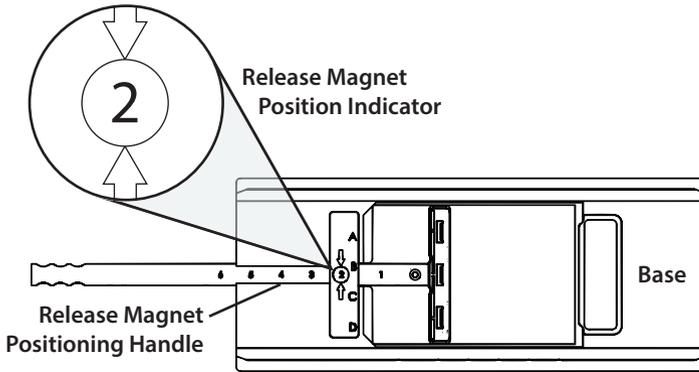


Figure 4: Release Magnet Positioning Handle (Initial Position)

3. Place the supplied 4-row microplate into the beveled recess of the base.

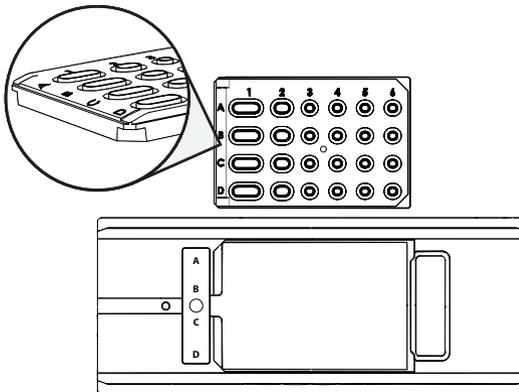


Figure 5: Orientation of 4-Row Microplate

Handle

1. Position the handle over a loaded bead capture strip rack.

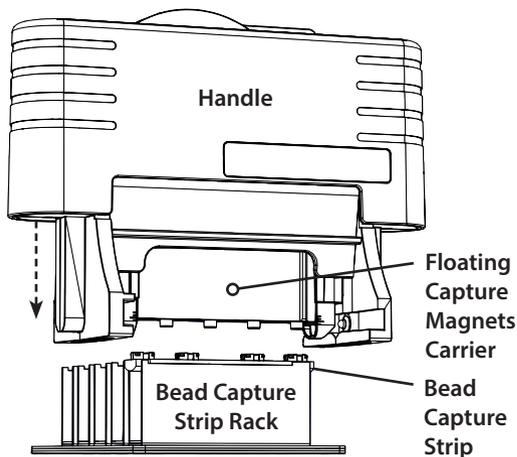


Figure 6: Loading the Bead Capture Strip

2. Align the floating capture magnets carrier over a bead capture strip.
3. Gently press down on the handle until the bead capture strip snaps into the floating capture magnets carrier.
4. Verify that the bead capture strip is properly seated and flush with the floating capture magnets carrier.
5. Slide the loaded handle onto the base.

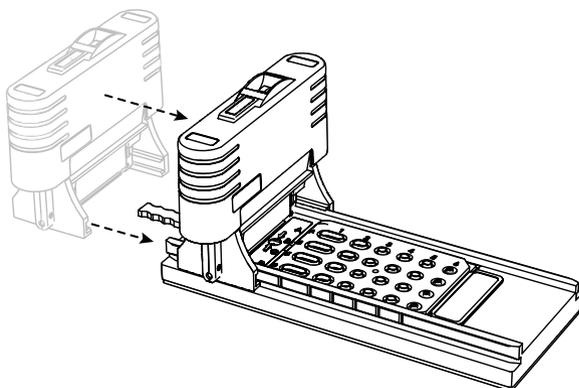


Figure 7: Handle Sliding onto Base

NOTE

Ensure that the side of the handle with the ball plunger faces the side of the base with notched indentations.

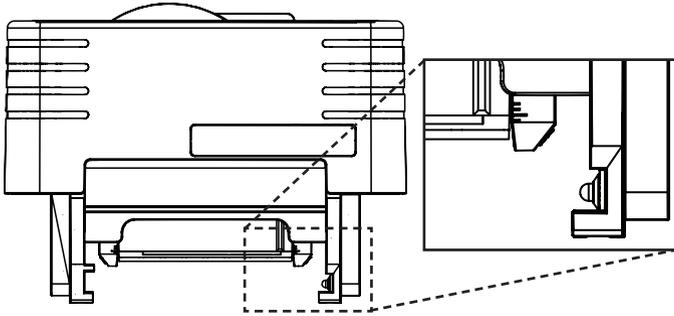


Figure 8: Close Up of Ball Plunger without Base

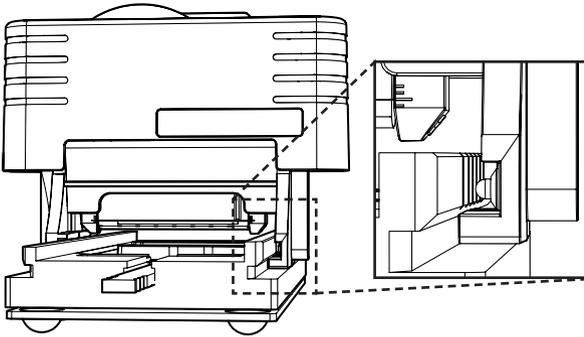


Figure 9: Close Up of Ball Plunger with Base and Notches

Basic Operation

Sample and Well Preparation

1. Prepare and thoroughly mix the desired paramagnetic bead and sample solution in a tube. Do not mix the sample directly on the 4-row microplate.

 **WARNING**

Observe safe laboratory practices when handling liquids. If working with biological samples or chemical substances, ensure that there is proper ventilation, and wear personal protective equipment (PPE), such as safety glasses, gloves, and protective clothing at all times.

NOTE

Follow the mixing recommendations of the bead or kit manufacturer before transferring the solution to the input well.

2. Transfer the sample solution to one of the input wells on the well plate.

Input, Wash, and Output Volumes

- The first well column (460–500 μL) was designed for input wells.
- The second (230–275 μL) and third (100–110 μL) well columns were designed for input or wash wells.
- The fourth and fifth (100–110 μL) well columns were designed for wash wells.
- The sixth (10–15 μL) well column was designed for output wells.

NOTE

Well volumes may vary because the formation of stable, prominent menisci may require the addition or subtraction of reagent, depending on the surface tension of the reagent used.

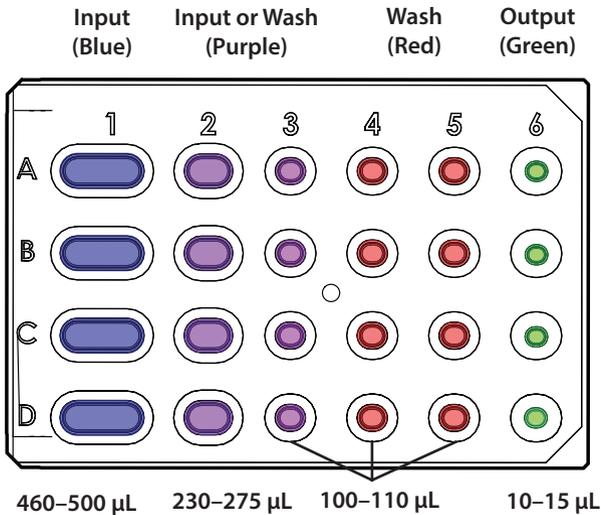


Figure 10: Input, Wash, and Output Well Locations

3. Fill each subsequent well with wash or reagent solution.

NOTE

Wells should have prominent menisci to ensure proper contact with the bead capture strip.

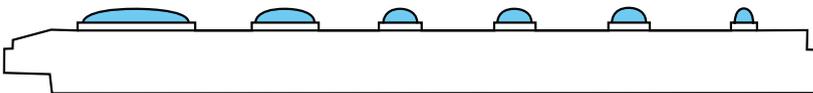


Figure 11: Prominent Menisci on the 4-Row Microplate

Bead Capture and Extraction

1. Slide the handle with the loaded bead capture strip over the center of Well Column 1 or the first input well until it clicks into place.

NOTE

Magnets contained in the floating capture magnets carrier will attract the paramagnetic beads in the input well.

Uncaptured paramagnetic beads will often color or cloud the liquid in the wells, so wait a few moments after the wells appear to be fully clear before advancing the capture magnet.

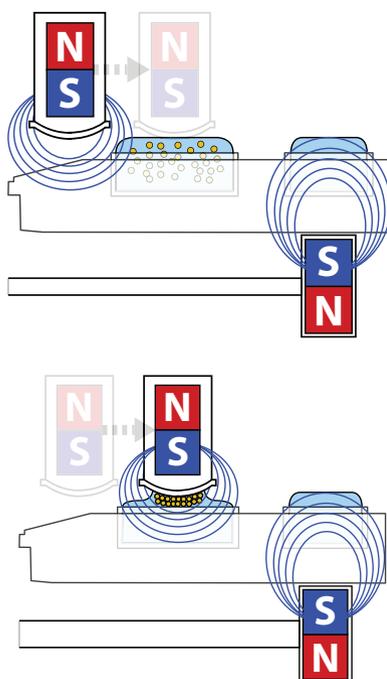


Figure 12: Input Well Bead Capture with Interior View of Magnetic Lines of Force

2. Move the handle with bead capture strip over the center of Well Column 2 or the first wash well until it clicks in place.

NOTE

The release magnet will force the capture magnets in the floating capture magnets carrier to retract and allow the paramagnetic beads to drop gently into the wash well.

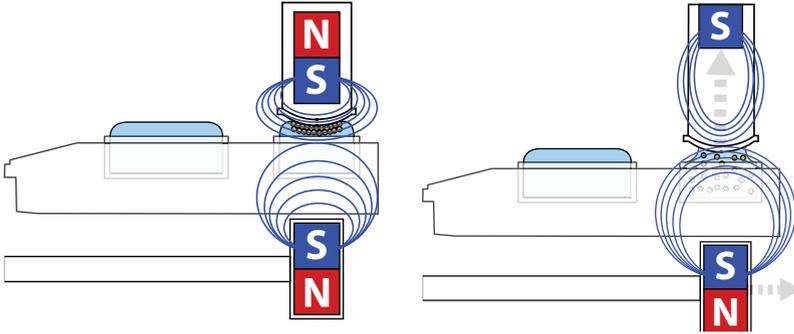


Figure 13: Magnet Retraction over Wash Well

3. Advance the release magnet positioning handle to the next well column until it clicks in place.

NOTE

The magnets in the floating capture magnets carrier will engage and attract the paramagnetic beads to the bead capture strip.

4. Move the handle with bead capture strip over the center of the next well column until it clicks in place.

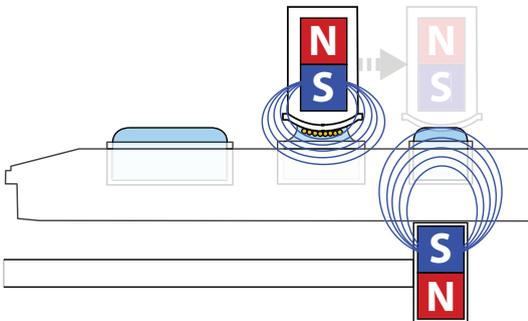


Figure 14: Paramagnetic Beads Recaptured

- Repeat steps 3 and 4 for the desired number of washes until the paramagnetic beads release into the output well.
- Slide the handle back to the previous wash well once the output well is reached.
- Use a pipette to remove the final purified paramagnetic bead and analyte solution from the output well.
- Press the **EJECTION** button to eject the bead capture strip.

NOTICE

Discard paramagnetic beads, bead capture strips, and 4-row microplates after use. Reuse of consumable plastics may result in contamination and the plastics may not function properly through multiple uses. Observe safe laboratory practices when disposing biological samples or chemical substances.

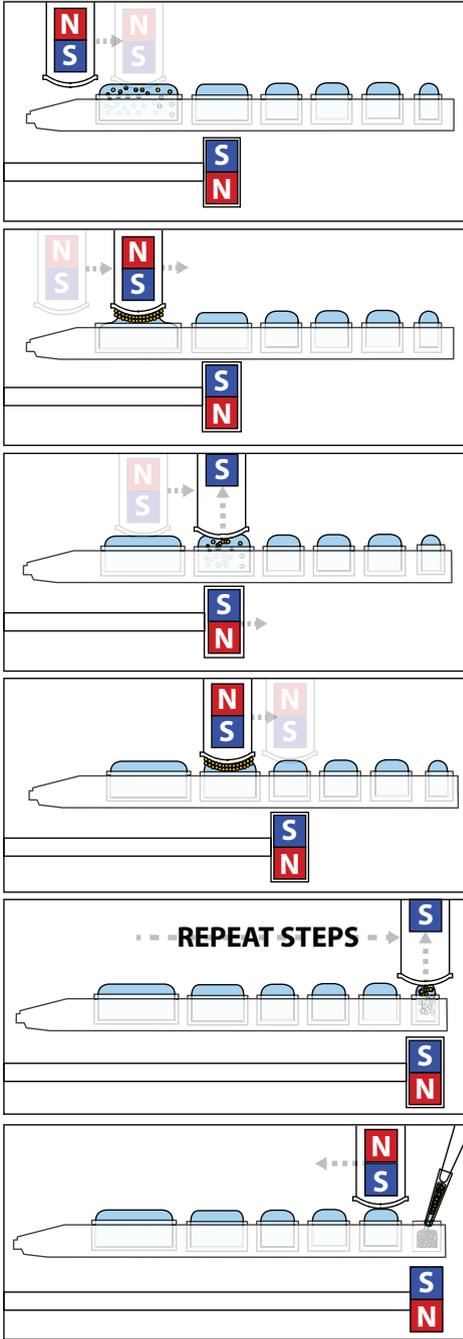


Figure 15: Full Steps for Extraction

Advanced Operation

EXTRACTMAN® is shipped from the factory preset at a height that should be suitable for most experiments. Should you need to adjust the height of the device to accommodate your sample preparation protocol, please follow these instructions.

NOTE

The magnets in the floating capture magnets carrier will engage and attract the paramagnetic beads to the bead capture strip.

Before you begin, ensure that EXTRACTMAN is clean and free of biological and chemical contaminants. Wear proper protective equipment (gloves, etc.) if needed.

Adjust the Collection Height

Adjust the collection height of EXTRACTMAN® to ensure proper contact with the menisci in the wells.

1. Slide the handle out from the base.
2. Press the **EJECTION** button to eject the bead capture strip.

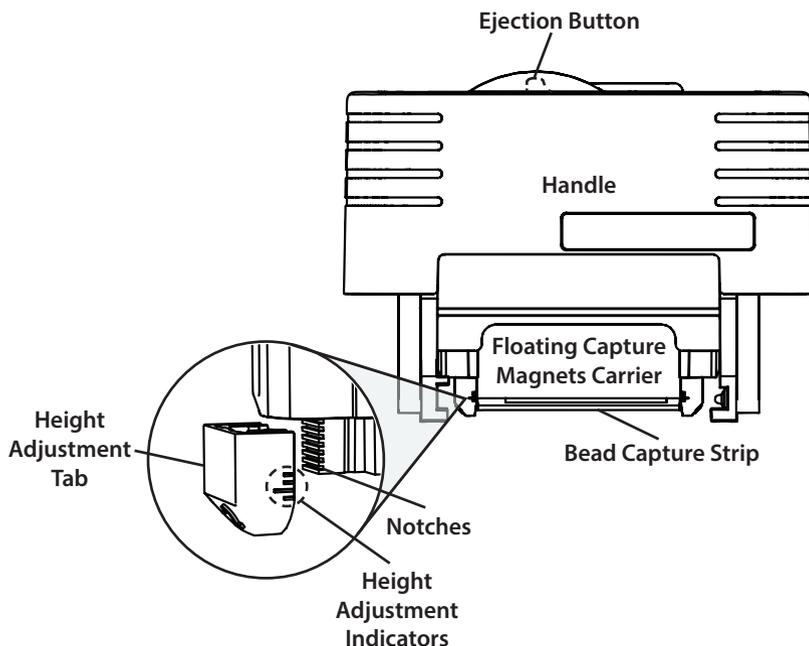


Figure 16: Handle Diagram with Views of Floating Capture Magnets Carrier Components

3. Grip the floating capture magnets carrier with one hand and retract it fully into the handle. Hold it in place.

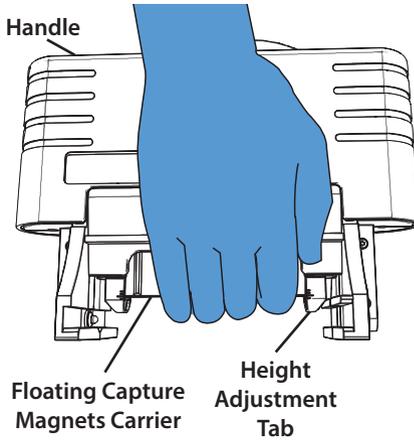


Figure 17: Gripping the Floating Capture Magnets Carrier

4. Use a flathead screwdriver to pry the height adjustment tab away from the floating capture magnets carrier on one side. Repeat this step for the other side.

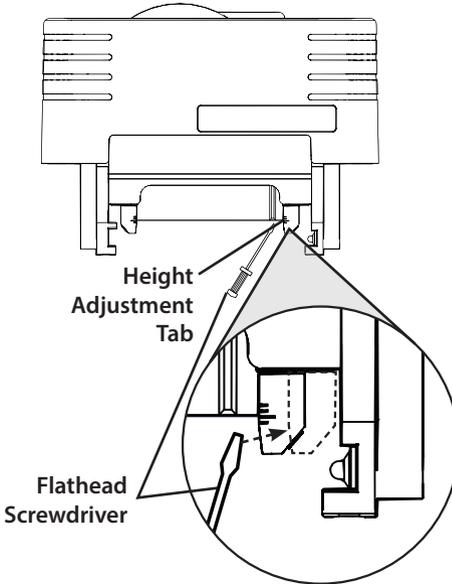


Figure 18: Removing the Height Adjustment Tab

5. Grip the floating capture magnets carrier with one hand and hold it down and away from the handle. Hold it in place.

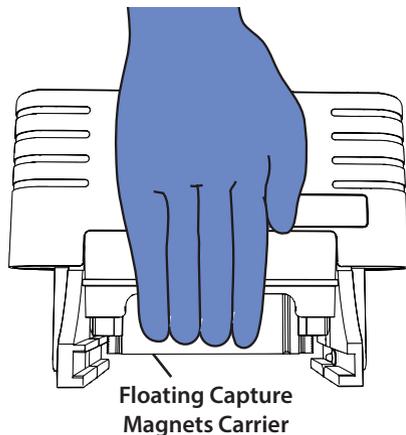


Figure 19: Holding Down the Floating Capture Magnets Carrier

6. Align one height adjustment tab at the desired height with the notches on the side of the floating capture magnets carrier.

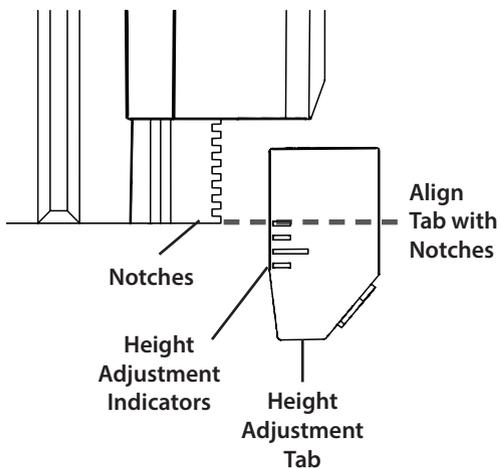


Figure 20: Close Up View of Height Adjustment Tab

7. Use a flathead screwdriver to push the height adjustment tab into the notches in the floating capture magnets carrier. Repeat steps six and seven for the other tab.

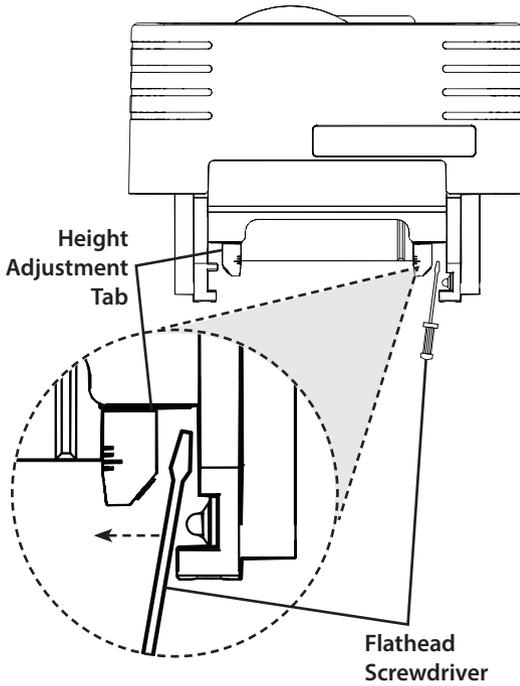


Figure 21: Installing the Height Adjustment Tab

8. Replace the bead capture strip. Refer to [Handle](#) on page 14.

Bead Size and Optimal Operation

The size of paramagnetic beads affects their time to pellet, suspension dynamics, and performance.

Adjust Protocols for Large Paramagnetic Beads

Larger paramagnetic beads exert greater magnetic force than smaller beads, resulting in a faster time to pellet. This dynamic may cause larger paramagnetic beads to pull off the bead capture strip prematurely. This can be avoided by raising the height of the bead capture strip. Refer to [Adjust the Collection Height](#) on page 22.

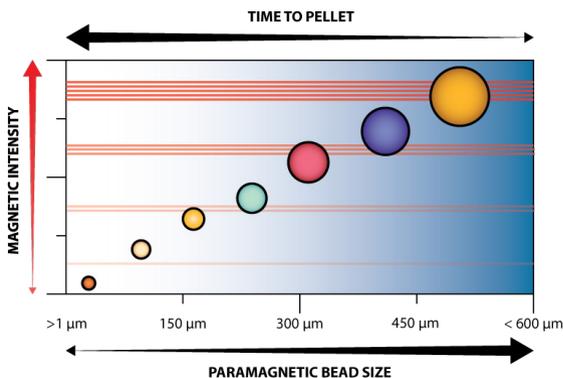


Figure 22: Magnetic Intensity and Paramagnetic Bead Size

Adjust Protocols for Small Paramagnetic Beads

Smaller paramagnetic beads exert less magnetic force than larger beads, resulting in a slower time to pellet. This dynamic may result in longer capture times. Gilson recommends waiting a few moments after the bead discoloration in the wells has cleared before advancing to the next well column.

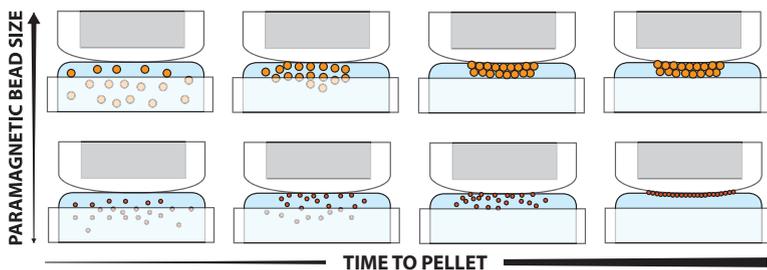


Figure 23: Paramagnetic Bead Size and Time to Pellet

Analyte Collection Without Beads

1. Perform the steps provided for **Basic Operation** on page 16.
2. Move the release magnet positioning handle back to the previous well column.

NOTE

The magnets in the floating capture magnets carrier will engage and attract the paramagnetic beads to the bead capture strip.

3. Expose the output well.
4. Use a pipette to remove the analyte without the paramagnetic beads from the well.

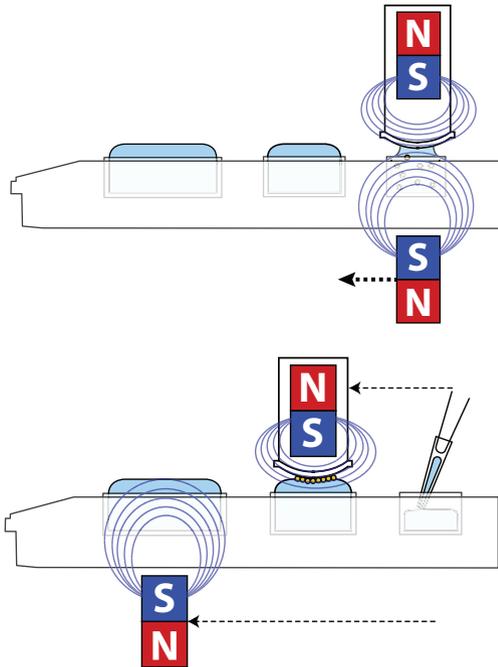


Figure 24: Alternative Analyte Removal

Adapt Protocols

Ensure Protocol Compatibility

Many paramagnetic bead-based protocols can be adapted for EXTRACTMAN®, including sample preparation (RNA, DNA, proteins, whole cells, etc.), negative selection assays, and library preparation, often with improvements to analyte yield.

Before adapting protocols, ensure that they meet all of the minimum requirements described below:

- Protocol uses para- or ferromagnetic beads to bind specific analytes.
- Paramagnetic beads are washed multiple times with one or more reagent.

NOTE

EXTRACTMAN® has not been tested with nano-particles and may not have strong enough magnetic fields to collect them.

Determine Work Flow

Most protocols adapted for EXTRACTMAN® will follow the same bind, wash, and elute work flow as defined in the original kit or protocol; however, because EXTRACTMAN® uses a 4-row microplate with pre-defined volumes, it may be necessary to adjust volumes for sample input, to combine, divide, or add reagent volumes to wells for washes or elutions, or to add an additional 4-row microplate to complete a protocol that requires additional washes. The number of downstream washes available on the 4-row microplate will be determined by which input well is used.

Adjust Volumes

Gilson recommends using the following volumes for sample, wash, and elution wells.

NOTE

Well volumes may vary because the formation of stable, prominent menisci may require the addition or subtraction of reagent, depending on the surface tension.

Volume Recommendations

Well Type	Volume
Large Sample	460 to 500 μL
Medium Sample	230 to 275 μL
Small Sample	100 to 110 μL
Wash	100 to 110 μL
Elute	10 to 15 μL

If the bind, wash, or elution volumes in the original protocol (i.e., the one being adapted) do not match the pre-defined well volumes in the 4-row microplate, distribute those volumes equally to the appropriate wells and add reagent to achieve the appropriate volume. If necessary, progressively add 5 μL of reagent to create well-defined menisci.

NOTE

Water-based solutions may form a meniscus from the interior wall of the well, whereas alcohol- or detergent-based solutions may form a meniscus across the top edge of the well. Both types of menisci are normal and will not affect the operation of EXTRACTMAN®.

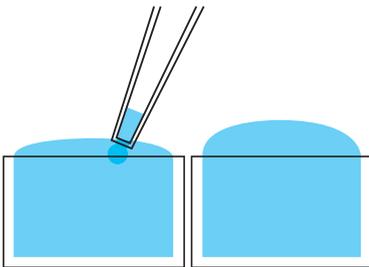


Figure 25: Adjusting Menisci for Water-Based Solutions

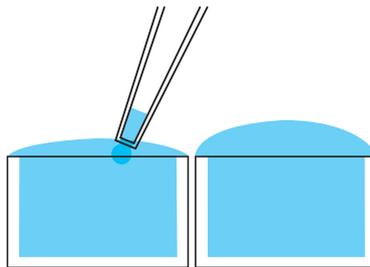


Figure 26: Adjusting Menisci for Alcohol- or Detergent-Based Solutions

Optimize Washes

EXTRACTMAN® washes are often more efficient than traditional tube-based washes. If required, two traditional washes may be replaced with a single EXTRACTMAN® wash, often with no decrease in performance.

If more washes are needed than there are available well columns, a second 4-row microplate may be used to complete additional wash steps. To accomplish this, slide the EXTRACTMAN® handle off the end of the base and exchange the 4-row microplate.

NOTE

Exchange the 4-row microplate quickly to reduce evaporation.

Determine Compatibility

Buffer Compatibility

The buffers in the table below have been tested successfully with EXTRACTMAN®; however, this table is not intended to be a comprehensive list of compatible buffers.

Compatible Buffers

<i>Buffer Type</i>	<i>Concentration</i>
Detergents (Up to 1%)	Triton™X100
	MP40
	Tween®-20
Salts	Any Concentration
Alcohol	Up to 70%

Bead Compatibility

EXTRACTMAN® is compatible with MagneSil®, Dynabead®, Agencourt DNAdvance™, and the majority of commercial bead types.

Analyte Compatibility

Nucleic acids (DNA and mRNA) and proteins, including antibodies, have been isolated with EXTRACTMAN®. DNA and mRNA isolates were suitable for downstream qPCR and RT-qPCR and quantification with a Qubit® spectrophotometer and kit. Genomic DNA has been isolated from cultured mammalian cells and plant tissue. Protein purification has been achieved using protein G-coupled beads to immobilize an antibody or streptavidin functionalized beads to immobilize a biotinylated antibody. In both cases, the captured antibody was used to isolate the desired antigen protein. Protein isolates were identified and yield was semi-quantitatively measured using silver-stained gels or western blots.

Determine Paramagnetic Bead Quantity

Gilson recommends using the paramagnetic bead quantities prescribed by the manufacturer. Greater quantities of paramagnetic beads will increase capture capacity, but may reduce purity and require more washes.

Test the Protocol

Follow the steps in [Basic Operation](#) on page 16 to confirm that the protocol has been successfully adapted to EXTRACTMAN®.

Example Protocol Adaptations

To clarify the protocol adaptation process, consider how the following sample protocols are adapted to EXTRACTMAN®.

Example Protocol 1

1. Resuspend paramagnetic beads in a vial for 30 seconds.
2. Add target antibody to 500 μL of binding solution in a tube.
3. Transfer 100 μL (3 mg) of paramagnetic bead solution to binding solution and incubate for 10 minutes.
4. Place the tube on a magnet rack and remove the supernatant.
5. Wash the paramagnetic beads three times using 400 μL washing reagent for each wash. Separate on the magnet rack between each wash, remove the supernatant, and resuspend in 400 μL washing reagent in a separate, clean tube.
6. Place the tube on a magnet rack and remove the supernatant.
7. Add 20 μL of elution reagent to the tube and resuspend with gentle pipetting.
8. Place the tube on a magnet rack and remove the supernatant.

Example Protocol 1 Adapted for EXTRACTMAN®

1. Resuspend paramagnetic beads in a vial for 30 seconds.
2. Add target antibody to 500 μL of binding solution in a tube.
3. Transfer 100 μL (3 mg) of paramagnetic bead solution to the binding solution and incubate for 10 minutes to create the sample solution.
4. Transfer the sample solution equally (150 μL) to each row in Well Column 2 on the 4-row microplate.
5. Add 80 μL of binding solution to each row in Well Column 2. If necessary, add 5 μL of binding solution to create well-defined menisci.
6. Add 110 μL of washing reagent to each row in Well Columns 3, 4, and 5. If necessary, progressively add 5 μL of washing reagent to create well-defined menisci.
7. Add 15 μL of elution reagent to each row in Well Column 6.
8. Operate EXTRACTMAN® according to the instructions provided in **Basic Operation** on page 16.

Example Protocol 2

1. Resuspend paramagnetic beads in the vial for 30 seconds.
2. Add target antibody to 2.0 mL of binding solution in a tube.
3. Transfer 500 μL (15 mg) of paramagnetic bead solution to binding solution and incubate for 10 minutes.
4. Place the tube on a magnet rack and remove the supernatant.
5. Wash the paramagnetic beads six times using 300 μL washing reagent for each wash. Separate on the magnet rack between each wash, remove supernatant, and resuspend in 300 μL washing reagent in a separate, clean tube.
6. Place the tube on a magnet rack and remove the supernatant.
7. Add 50 μL of elution reagent to tube and resuspend with gentle pipetting.
8. Place the tube on a magnet rack and remove the supernatant.

Example Protocol 2 Adapted for EXTRACTMAN®

1. Resuspend paramagnetic beads in the vial for 30 seconds.
2. Add target antibody to 2.0 mL of binding solution in a tube.
3. Transfer 500 μL (15 mg) of paramagnetic beads solution to binding solution and incubate for 10 minutes to create the sample solution.
4. Transfer 460 μL of sample solution to each row in Well Column 1 on the 4-row microplate. If necessary, progressively add 5 μL of binding solution to create well-defined menisci.
5. Transfer the remaining sample solution equally (165 μL) to each row in Well Column 2 on the 4-row microplate.
6. Add 65 μL of binding solution to each row in Well Column 2 on the 4-row microplate. If necessary, progressively add 5 μL of binding solution to create well-defined menisci.
7. Options for Wash Steps
 - If combining wash steps according to [Optimize Washes](#) on page 30, add 110 μL of washing reagent solution to each row in Well Columns 3, 4, and 5. If necessary, progressively add 5 μL of washing reagent to create well-defined menisci.
 - If not combining wash steps, add 110 μL of washing reagent solution to each row in Well Columns 3, 4, and 5, and then slide the handle off the end of the base. Place a new 4-row microplate and refill the wash wells with the 110 μL of washing reagent. If necessary, progressively add 5 μL of washing reagent to create well-defined menisci.

NOTE

Exchange the 4-row microplate quickly to reduce evaporation.

8. Add 15 μL of elution reagent to each row in Well Column 6.
9. Operate EXTRACTMAN® according to the instructions provided in [Basic Operation](#) on page 16.

Immunoprecipitation (IP) Protocol for EXTRACTMAN® using *E. coli* whole cell extract (WCE) and Epitope-tagged Enhanced Green Fluorescent Protein (etEGFP)

Reference for mAb 8RB13-epitope tag and mAb 1GFP52: Stalder et al. (2011) *Protein Expr. Purif.* 77: 26-33.

Materials

- Beads
 - Protein-G Dynabeads® (Thermo Fisher) or Protein-G beads from other sources
- Sample
 - *E. coli* WCE containing 8RB13-epitope-tagged enhanced green fluorescent protein (etEGFP)
- Target Specific Antibody
 - mAb 8RB13 (about 1 mg/mL): Antibody reactive with the epitope-tag for immunoprecipitation
- Buffer
 - Tris-Buffered Saline + 0.01% Triton X-100
- Equipment
 - EXTRACTMAN®
 - Magnetic rack for microtubes
 - PIPETMAN® (P20, P200, P1000)
- Consumables
 - EXTRACTMAN® 4-row microplate
 - EXTRACTMAN® bead capture strips
 - 1.5 mL microtubes
 - Pipette tips

Load Beads with Antibody

1. Thoroughly resuspend paramagnetic beads.
2. Immediately transfer 20 μL of the paramagnetic bead slurry (20 μg of paramagnetic beads) into a 1.5 mL microtube (one tube for each sample).
3. Add 200 μL of buffer to the tube containing the paramagnetic beads and thoroughly mix.
4. Place the tube on the magnetic rack, and then wait for the paramagnetic beads to collect on the side wall of the tube.
5. Remove the buffer.
6. Repeat steps three through five.
7. Add 190 μL of buffer to each tube.
8. Add 10 μL of mAb 8RB13 and thoroughly mix.
9. Incubate at room temperature for 20 minutes. Thoroughly mix every three minutes.
10. Place the tube on the magnetic rack, and then wait for the paramagnetic beads to collect on the side wall of the tube
11. Remove the buffer-containing mAb and save, if desired.
12. Add 200 μL of buffer and thoroughly mix.
13. Place the tube on the magnetic rack. Remove the buffer.
14. Repeat steps three through five.

Set Up Immunoprecipitation

1. Add 300 μL of WCE (sample) to the microtube containing the mAb-bound beads and incubate on ice for 30 minutes, thoroughly mix every five minutes.
2. Place a 4-row microplate on the EXTRACTMAN[®] base.
3. Position the release magnet under the first wash well column (Well Column 3).
4. Resuspend the beads and transfer 250 μL of the IP reaction to the 230 μL input well column (Well Column 2) on the 4-row microplate.

NOTE

Well volumes may vary because the formation of stable, prominent menisci may require the addition or subtraction of buffer, depending on the surface tension of the buffer used. In this case, the 230 μL input well is overfilled to create prominent menisci.

5. Load the three wash wells (Well Columns 3, 4, and 5) on the 4-row microplate with 100 μ L of buffer. Make sure that all wells have a convex meniscus of about 2 mm.
6. Secure the bead capture strip to the handle of the EXTRACTMAN® and move the handle into place on the EXTRACTMAN® base. Refer to **Operation** on page 16.
7. Move the handle over the 230 μ L (Well Column 2) to collect the beads from the WCE sample. Hold briefly until all of the paramagnetic beads are recovered.
8. Move the handle to the first wash well column. The beads will fall to the bottom of the first wash well column. Hold briefly until all of the paramagnetic beads have dropped.
9. Repeat steps 7 and 8 for the remaining washes.
10. Move the release magnet to the output (elution) well column (Well Column 6). The beads will rise from the bottom of the final wash well column (Well Column 5) to the bead capture strip. Hold briefly until all of the paramagnetic beads are recovered.
11. Move the handle to the output well column. The beads will fall to the bottom of the output well. Hold briefly until all of the paramagnetic beads have dropped.
12. Gently slide the handle off the base and eject the bead capture strip.
13. Recover the beads for analysis.
14. Perform analysis of the fractions as dictated by experimental design.

Troubleshooting

Troubleshooting Table

<i>Symptom/s</i>	<i>Possible Cause</i>	<i>Possible Solution</i>
Bubbles in the wells	Pipette mixing	Use a gentler mixing technique.
	Pipetting	Prewet pipette tips.
	NOTE	Bubbles typically do not affect the performance of EXTRACTMAN®.
Paramagnetic beads are not captured on the bead capture strip	Paramagnetic beads are stuck to the sides of the well	Mix the contents of the well to dislodge the paramagnetic beads.
		Hold the handle over the well for a longer period of time.
		Move the handle back and forth through the meniscus to help release the paramagnetic bead aggregate.
	Fluid is not making contact with the strip	Hold the handle over the well for a longer period of time.
		Lower the collection height. Refer to Adjust the Collection Height on page 22.
	Evaporation may have caused the well volume to become too low	Add fluid until a stable meniscus is formed. Because of the differences in surface tension, the curvature of the meniscus for each reagent may be different. Gilson recommends adding five percent of total volume until the meniscus holds a stable shape.

Troubleshooting Table, continued on page 39

<i>Symptom/s</i>	<i>Possible Cause</i>	<i>Possible Solution</i>
Paramagnetic beads are not captured by the strip (continued...)	Paramagnetic beads are too small, weakly magnetic, or slow to respond	Hold the handle over the well for a longer period of time.
		Paramagnetic beads may be too small to be compatible with EXTRACTMAN®. Use larger paramagnetic beads.
		Lower the collection height. Refer to Adjust the Collection Height on page 22.
	The capture magnets (visible through the transparent plastic) are not in the down position	Ensure that the release magnets are not positioned directly beneath the capture magnets. For the final output well, the release magnet can be pushed past the final well to allow the capture magnets to reengage.
Fluid is spilling over the edge of the wells	The handle is being slid too fast.	Slow down the slide speed.
	Too much fluid in the wells	Reduce fluid volume slightly.
	Collection height is too low	Raise the collection height. Refer to Adjust the Collection Height on page 22.
	Paramagnetic bead aggregate is too high	Reduce the quantity of the paramagnetic beads used for the assay.
		Raise the collection height. Refer to Adjust the Collection Height on page 22.
	Reagents contain surfactant or alcohols that have a low surface tension, making them prone to spillage	Reduce the volume.

Troubleshooting Table, continued on page 40

<i>Symptom/s</i>	<i>Possible Cause</i>	<i>Possible Solution</i>
Paramagnetic beads are releasing prematurely or falling outside of the well or into the well moat	Paramagnetic beads are too magnetically strong	Raise the collection height. Refer to Adjust the Collection Height on page 22.
	Paramagnetic bead aggregate is too high	Slide the release magnet forward to the next well column before advancing the handle, and then slide the release magnet back to the well column beneath the handle.
		Reduce the paramagnetic bead quantity.
Fluid remains on the bead capture strip	Samples are too protein rich	No action needed. The fluid should naturally dilute after the sample is taken through the wash steps.
Handle slides when moving the release magnet	Magnetic field interactions	Ensure that the handle is secured into one of the notches while moving the release magnet.
Release magnet slides while moving handle	Magnetic field interactions	Ensure that the release magnet is secured into one of the notches while moving the handle.
Contamination in the final elution well or low purity	Insufficient washing	Add additional wash wells.
		Add additional (5 to 10) capture and release steps during the wash and elution steps.
	Gel beads or porous beads	Add longer delays to each wash step.
		Add additional washes.
		Repeatedly capture and resuspend the paramagnetic beads five to ten times.

Troubleshooting Table, continued on page 41

<i>Symptom/s</i>	<i>Possible Cause</i>	<i>Possible Solution</i>
Paramagnetic beads are clumped together	Samples are sticky	Mix in the wash well.
		Slide the capture and release magnets back and forth, magnetically mixing the paramagnetic beads.
Strip doesn't eject	The floating capture magnet carrier is not in the downward position	Press up on the strip to reload the ejection mechanism and then press the EJECTION button again.
		Pull the strip off by hand by using the tabs along the sides.
EXTRACTMAN® base is sliding	The rubber feet are dirty	Clean the rubber feet with soap and water.
	The base is seated on top of a slippery surface	Make sure the base is positioned directly on the table.

Target Type and Optimal Operation

Certain target types, such as DNA, bacteria, and cells, may cause the paramagnetic beads to irregularly clump or stick to plastic components. This dynamic may require users to move the sliding handle back and forth over the well column to release the paramagnetic beads. Alternatively, if the beads are stuck to the well itself, Gilson recommends pelleting and resuspending the beads between the capture and release magnets.

Viscous Lysates and Analytes and Optimal Operation

Highly viscous lysates and analytes that result in gelatinous bead-analyte complexes (as in some gDNA kits) may result in inefficient bead movement and increased contaminant carryover.

Repair and Return Policies

Refer to the following information and then contact your local Gilson representative. Specific contact information can be found at www.gilson.com.

Before Calling Us

Your local Gilson representative will be able to serve you more efficiently if you have the following information:

- Serial number and model number of the instrument, located on the bottom of the base
- List of concise symptoms
- List of operating procedures and conditions you were using when the problem arose

Warranty Repair

Units covered under warranty will be repaired and returned to you at no charge. If you have any questions about applicability, contact your local Gilson representative.

Non-Warranty Repair

For out-of-warranty repairs, contact your local Gilson representative who will discuss service options with you and can assist in making arrangements to return the equipment, if necessary.

Return Procedure

Contact your local Gilson representative to obtain authorization before returning any Gilson equipment. To return a piece of equipment:

- Carefully pack the unit to prevent damage in transit. Check with your local Gilson representative regarding proper method of shipment. No responsibility is assumed by Gilson or your local Gilson representative for damage caused by improperly packaged instruments. Indicate the authorization on the carton and on the packing slip.
- Always insure for the replacement value of the unit.
- Include a description of symptoms, your name, address, phone number, and purchase order to cover repair costs, return and shipping charges, if your institution requires it.

Unit End-of-Life

When a unit reaches the end of its useful life, refer to www.gilson.com for directions and information on the end-of-life policy. This is in accordance with the European Union Directive 2002/96/EC on Waste Electrical and Electronic Equipment (WEEE).



Parts and Accessories

<i>Part Number</i>	<i>Description</i>
22100000	EXTRACTMAN® Starter Kit
22100007	Bead Capture Strips (x 25)
22100008	4-Row Microplates (x 25)
22000014	Height Adjustment Tab (x 2)