POROS™ 20 EP Media Immobilizing Ligand for Perfusion Immunoassay™ Technology

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

In this document

This document describes the procedures for covalently immobilizing ligands to POROS™ epoxide (POROS™ EP) media for use in ImmunoDetection™ (ID) Sensor Cartridges.

This document describes three methods for immobilizing ligand. The method you choose depends on the ID product you purchase, and your applications needs:

- If you purchased an ID Sensor Cartridge kit (contains packed sensor cartridge, buffers, and reagents)—Immobilize ligand directly on the packed cartridge.
- If you purchased an ID Sensor Cartridge (contains packed sensor cartridge)—Immobilize ligand directly on the packed cartridge.
- If you purchased an ID Self Pack™ kit and an ID Self Pack™ packing device (contains bulk media, buffers, empty cartridges, and the device needed to pack the cartridges)—Immobilize ligand on a batch of media before packing the cartridge, or pack the cartridge and immobilize ligand directly on the packed cartridge.

Note: We have extensively tested ligand immobilization on POROS™ EP media with protein ligands, particularly antibodies and antigens. While POROS™ EP media may be suitable for non-protein ligands, non-protein ligands have not been as extensively tested. If you have non-protein ligands that cannot be precipitated with salt, or that do not include reactive amine or sulfhydryl groups, you may want to use a BA ID Sensor Cartridge. The BA ID Sensor Cartridge contains immobilized streptavidin and can be used to immobilize any biotinylated ligand.

Related documents

You may receive two additional documents, depending on the product you purchase:

- ***ID Self Pack™ Packing Device Operating Instructions***—Refer to this document before immobilizing ligand, if you want to immobilize ligand on the packed cartridge. Refer to this document after immobilizing ligand, if you want to first immobilize ligand on a batch of media.
- ***Performing Unlabeled Perfusion Immunoassays***—Refer to this document after immobilizing ligand and packing the cartridge.
- ***Preparation of ImmunoDetection™ (ID) Cartridges: Guidelines for Immobilization of Antibodies***—Refer to this document before immobilizing antibody.

Ligand immobilization on POROS™ EP media

Epoxide conjugation is a general-purpose chemistry used to covalently immobilize antigens, antibodies, receptors, or receptor binding proteins. Epoxide groups react with amino, sulfhydryl, and hydroxyl groups to form a stable covalent bond.

POROS EP

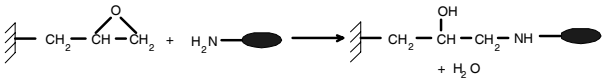

Fig. 1 Epoxide immobilization

High salt concentrations (1.0 to 1.5 M Na_2SO_4) are used to precipitate ligand onto the surface of the POROS™ EP media particle, creating a high concentration of ligand at the reactive surface. This technique does not disturb protein structure and maintains a high level of biological activity. It is biochemically similar to:

- The use of ammonium sulfate precipitation to concentrate and partially purify antibodies
- Hydrophobic interaction chromatography (HIC)

Highest ligand coupling efficiencies are obtained when the salt concentration is just sufficient to bring the protein out of solution on the particle, not with the highest possible salt concentration. The methods presented in this document provide guidance in adjusting salt concentrations to the optimum levels for your ligand.

CAUTION! Salt concentrations above 1M can damage instrument valves, syringes, and pumps. Flush your instrument thoroughly with water after using high-salt buffers.

Note: Use Na_2SO_4 buffers instead of NH_4SO_4 buffers. Ammonium ions may compete with amines on the ligand for coupling. Also, high-salt buffers can settle and crystallize during storage. Mix buffers well before use.

Most protein ligands will be successfully immobilized on the POROS™ EP media surface in the salt concentrations of the buffers provided with the kit. If your ligand is not an antibody, or if you are unsure of the salt levels required to immobilize your ligand, see "Determining salt concentration needed" on page 6.

Materials

Materials provided (buffers not provided if you purchase a sensor cartridge only)

- EP ID Sensor Cartridge, 2.1 mmD/30 mmL (in ID Sensor Cartridge Kit)
- Empty cartridges and bulk POROS™ EP media (in ID Self Pack™ Kit)
- Loading buffer, 1 pack, sufficient for 1 liter of 150 mM NaCl and 10 mM phosphate, pH 7.4
- Elution buffer, 1 pack, sufficient for 1 liter of 150 mM NaCl, pH 2
- EP high-salt buffer, 1 pack sufficient for 100 ml of 1.5 M Na2SO4 in 100 mM phosphate, pH 8.5–9.0
- EP low-salt buffer, 1 pack, sufficient for 100 ml of 0.5 M Na2SO4 in 100 mM phosphate, pH 8.5–9.0

Materials needed but not provided

- Ligand at a final concentration of 0.5 to 1 mg/ml (0.5 to 3 mg total protein per ID sensor cartridge).
- 1 ml pipettes and pipetting aids, test tubes
- 12 M HCl
- NaOH
- INTEGRAL™ Micro-Analytical Workstation, BioCad™ Workstation, BioCad™ RPM™ System, or BioCad™ Sprint™ System. If these systems are not available, use an HPLC system with 2-solvent capability, UV detector, and injection valve. Pump must be able to switch between two buffers at a flow of 0.5 to 6.0 ml/min.
- HPLC sample loop twice the volume of the largest injection.
- For recycling method—15 ml test tube or a 10 ml Luer-Lok™ syringe and Luer-to-1/4-28 connector for connecting the syringe to low pressure tubing.
- For bulk method—50 ml screw-cap test tube, rocker or shaker for 50 ml test tube, and 10–20 µm sintered glass funnel.

Note: A fitting adapter kit is available from **thermofisher.com** to connect the ID sensor cartridge to metric FPLC™ systems and to 1/4-28 thread fitting systems. See "Accessories, spare parts, and ordering information" on page 7.

Selecting the immobilization method

There are three methods for immobilizing your ligand:

- Recycling (online method)
- Injection (online method)
- Bulk (offline method)

Suggestions:

- If you have a limited supply of ligand, use the recycling method. All immobilized ligand will be available on the cartridge and any excess ligand can be recovered.
- If it is difficult to recycle on your instrument, use the injection method.
- If you want to make several cartridges with the same batch of ligand, use the bulk method (available with ID Self Pack™ only).

Table 1 Comparison of immobilization methods

Method	Recycling	Injection	Bulk
Use with	• ID cartridge • ID kit • ID Self Pack™	• ID cartridge • ID kit • ID Self Pack™	ID Self Pack™ only
Instrument requirements	• Low system volume • Total volume of recycling loop < 2 ml • Can easily be flushed to remove salt and protein	Any HPLC or FPLC	None required for immobilization
Number of cartridges	1	1	Many from the same batch of media
High capacity cartridges	Yes	Yes	Yes
Time to make cartridge	About 18 hr	About 18 hr	About 18 hr
Instrument time	About 1.5 hr	About 1.5 hr	Only time for packing cartridges
Recovery of unbound ligand	Yes	No	Possible, but in a very dilute solution
Monitoring of ligand capture	Yes	Yes	No
Efficiency of ligand use	Efficient	Efficient	Less efficient, because some media wasted during packing
HPLC pumps	Exposed to 1.1 M salt and protein	Exposed to 1.5 M salt	Not used

Recycling method

The recycling method produces the highest level of ligand loading in ID sensor cartridges and is easily automated on the INTEGRAL™ Micro- Analytical Workstation, the BioCad™ Workstation, the BioCad™ RPM™ System, or the BioCad™ Sprint™ System.

The total volume of the recycling loop on your instrument should be less than 2 ml to prevent sample dilution.

During a recycling method, the following occurs:

- Buffer containing ligand and Na2SO4 is recycled through the ID sensor cartridge for about 1.5 hours.
- Salt concentration is gradually increased until the ligand precipitates out of solution onto the POROS™ EP media in the ID sensor cartridge. Because the UV detector is plumbed in the recycling loop, you can easily monitor the level of unbound ligand in the recycling solution.
- When ligand is precipitated on the cartridge, you remove the cartridge from the instrument, cap the cartridge, and incubate the cartridge at room temperature for 16 to 24 hours. During this incubation, the ligand covalently immobilizes on the ID sensor cartridge.
- After incubation, you reinstall the cartridge and flush it with loading buffer.

See "Recycling method" on page 4 for more information.

Injection method

You can perform the injection method on any HPLC or FPLC system.

During an injection method, the following occurs:

- 20 µl samples of the ligand are injected into a flowing stream of 1.5 M salt.
- The small sample volume mixes with the salt mobile phase and is captured on the cartridge.
- Additional injections are made until the entire sample is loaded.

- A decreasing salt gradient is run until the salt concentration is low enough to elute the ligand.
- When the ligand begins to elute, you stop flow to the cartridge, remove the cartridge from the instrument, cap the cartridge, and incubate the cartridge at room temperature for 16 to 24 hours. During this incubation, the ligand covalently immobilizes on the ID sensor cartridge.
- After incubation, you reinstall the cartridge and flush it with loading buffer.

See "Injection method" on page 5 for more information.

Bulk method

The bulk method of immobilization allows you to prepare a set of ID sensor cartridges that contain the same batch of ligand:media preparation.

During a bulk method, the following occurs:

- Ligand is suspended in 0.75 M salt buffer.
- POROS™ EP media is added to the solution and mixed.
- Salt concentration is increased to 1.1 M.
- The mixture is rocked and incubated at room temperature for 16 to 24 hours. During this incubation, the ligand covalently immobilizes on the POROS™ EP media.
- The immobilized media is filtered in a sintered glass funnel and can be packed in an ID Sensor Cartridge.

See "Bulk method" on page 6 for more information.

Preparing buffers

The ID Sensor Cartridge Kit and ID Self Pack™ Kit provide sufficient buffer materials to immobilize the ligand on the cartridge and to run several capture/elution assays.

If you need additional buffer, or if you purchased a packed cartridge only, prepare the buffers according to the formulations given in this section.

Loading buffer (150 mM NaCl 10 mM phosphate pH 7.4)

To prepare the loading buffer supplied with the kit:

1. Dissolve the contents of pack in 0.9 liter deionized H_2O.
2. Check the pH and adjust to pH 7.4 with HCl or NaOH as necessary.
3. Bring the volume to 1 liter.
4. Filter the solution with a 0.22 μm filter before use.

Note: Filter the buffer daily if it is used over several days. Store unused loading buffer at 2 to 8°C.

Elution buffer (12 mM HCl + 150 mM NaCl pH ~2.0)

To prepare the elution buffer supplied with the kit:

1. Dissolve the contents of pack in 0.9 liter deionized H_2O.
2. Add 1 ml of concentrated HCl (12 M) for a final concentration of 12 mM HCl.
3. Check the pH and verify that it is near pH 2.0 (between pH 1.8 and pH 2.2). Adjust with HCl or NaOH as necessary.
4. Bring the volume to 1 liter.
5. Filter the solution with a 0.22 μm filter before use.

Note: Filter the buffer daily if it is used over several days. Store unused elution buffer at 2 to 8 °C.

EP high-salt buffer (1.5 M Na_2SO_4 in 100 mM phosphate, pH 8.5–9.0)

To prepare the EP high-salt buffer supplied with the kit:

1. Dissolve the contents of the package in 90 ml deionized H_2O.
2. Adjust the pH to 8.5–9.0 with NaOH or HCl as necessary.
3. Bring the volume to 100 ml.
4. Filter the solution with a 0.22 μm filter before use.

Note: Store at room temperature. Mix well before each use, since crystallization of the salt may not be readily apparent. If salt has crystallized out of solution, warm on low heat while stirring on a heat/stir plate until the salt goes back into solution.

EP low-salt buffer (0.5 M Na_2SO_4 in 100 mM phosphate, pH 8.5–9.0)

To prepare the EP low-salt buffer supplied with the kit:

1. Dissolve the contents of the package in 90 ml deionized H_20.
2. Adjust the pH to 8.5–9.0 with NaOH or HCl as necessary.
3. Bring the volume to 100 ml.
4. Filter the solution with a 0.22 μm filter before use.

Note: Store at room temperature. Mix well before each use, since settling of the salt may not be readily apparent. If salt has crystallized out of solution warm on low heat while stirring on a heat/stir plate until the salt goes back into solution.

Preparing ligand

Make sure ligand:

- Is as pure a ligand solution as possible. All proteins in the solution will be conjugated.
- Is in a buffer free of amino groups. Do not use Tris buffer or a solution containing sodium azide. If your ligand is in an unsuitable buffer, exchange it into loading buffer.
- Is at a final concentration of 0.5 to 1 mg/ml.
- Provides 0.5 to 3 mg total protein per ID sensor cartridge.

Connecting the sensor cartridge

For recycling and injection methods, connect the sensor cartridge to your instrument as described below.

Note: Use the E-Z Grip™ fittings supplied with the sensor cartridge. Do not use standard stainless steel fittings that require tightening with a wrench. Over-tightening can strip the threads of the ID sensor cartridge.

1. Remove the end plugs from the sensor cartridge and save them for future storage of the cartridge.
2. Slip an E-Z Grip™ compression fitting over the end of the tubing.
3. Slip a PEEK ferrule over the end of the tubing. You can place either end of the ferrule toward the cartridge. Make sure at least 1/6-inch of tubing extends from the ferrule.
4. Bottom the tubing in the end fitting of the cartridge. Finger-tighten the compression fitting. Do not over-tighten.

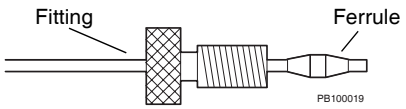

Fig. 2 Connecting E-Z Grip™ fittings

Recycling method

Plumbing the instrument

The total volume of the recycling loop should be less than 2 ml. A larger volume will dilute the sample.

1. Plumb the inlet line of the pump to one of the following (Figure 3 on page 4):
   - 15 ml test tube so that it draws from the bottom of the tube.
   - Outlet of a 10 ml syringe using a Luer-to-1/4 28 adapter. Remove the plunger and mount the syringe vertically.
2. Plumb the pump outlet line to the UV/Vis detector inlet. Plumb the UV detector to the ID sensor cartridge inlet.
3. Connect a line to the cartridge outlet that you can move manually between the test tube or syringe and waste.

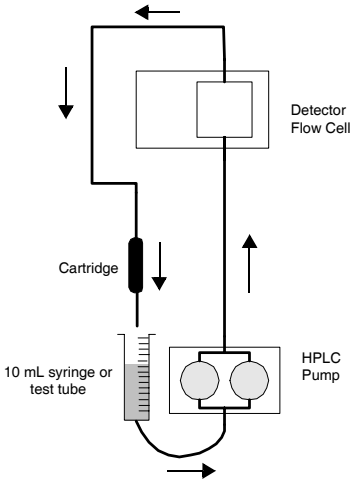

Fig. 3 Recycling method plumbing

Preparing the ligand

You can use any volume of ligand because the salting-out procedure concentrates ligand on the cartridge. However, volumes of a few milliliters are easiest to prepare.

Mix 1 part ligand solution with 1 part EP high-salt buffer. Add the buffer slowly while mixing to avoid high concentrations of salt that may precipitate ligand.

The ligand is now suspended in a concentration of 0.75 M Na2SO4.

Preparing instrument and cartridge for immobilization

1. Fill the test tube or syringe with EP low-salt buffer. Direct the cartridge outlet to waste.
2. Flush the ID sensor cartridge at 1 to 3 ml/min for 2 minutes, or until you no longer observe spikes in the UV signal that indicate bubbles in the tubing or flow cell.
3. Place 2 ml of EP low-salt buffer in the test tube or syringe and recycle this solution at 1 to 3 ml/min. Check to make sure that the fluid level remains constant over a period of 3 minutes.
4. Set the UV detector at 280 nm and zero it.

   Note: If your ligand absorbs poorly at 280 nm or has unusual chromogenic properties, select an appropriate wavelength for monitoring.
5. Stop the pump and replace the cartridge with a union.
6. Remove all but 0.3 ml of the EP low-salt buffer from the test tube or syringe using a pipette. Leave 0.3 ml of solution in the test tube or syringe to ensure that air bubbles are not drawn into the pump inlet line.

Immobilizing the ligand using the recycling method

1. Pipette the prepared ligand solution into the test tube or syringe.
2. Resume pumping at 1 to 3 ml/min for 5 minutes. The ligand solution should completely mix with the EP low-salt buffer already in the system.
3. Observe the UV signal until you see a stable reading of 280 nm (or the selected wavelength), showing an equilibration of the recycling mobile phase with the cartridge. Note the absorbance.
4. Stop the pump, and reinstall the cartridge.
5. Resume recycling the solution. Much of the ligand may precipitate on the EP ID sensor cartridge even at this low level of salt. Observe the UV signal until you see a stable reading of 280 nm (or the selected wavelength).
6. Add 1 ml of EP high-salt buffer to the test tube or syringe, recycle for 3 to 5 minutes, and allow the solution to re-equilibrate at this increased salt concentration.
7. Repeat step 6 one-to-four more times, until the UV absorbance at 280 nm (or the selected wavelength) is approximately 10% of the absorbance noted in step 3. For example, if you originally noted an absorbance of 1.2 AU, repeat step 2 until you see an absorbance of 0.12 AU.

   If absorbance does not decrease to 10% of the original absorbance, repeat step 6 until you see a minimal change in absorbance with each addition of EP high-salt buffer.

   Do not add excess salt. Add only enough salt to precipitate the ligand on the EP surface. If the absorbance of the recycling solution is below 10% of the original absorbance, do not add more salt.

   Note: The "10% of the absorbance noted" is intended as a general guide, which implies 90% of the starting ligand is on the cartridge surface. If you started with a total ligand near 3 mg, you may be near the saturation level of the cartridge and may not be able to reduce the unbound recirculating ligand to this level.
8. Allow the system to recycle at 1 to 3 ml/min for an additional 30 minutes.
9. Stop the pump. Remove the cartridge and cap it. Incubate the cartridge for 16 to 24 hours at room temperature.

   Note: Do not refrigerate the cartridge. Salt will precipitate and may damage the POROS™ particles.
10. Remove the solution from the test tube or syringe using a pipette.

    This solution contains unbound ligand that you can use in other applications.
11. Flush the system with water to remove salt and protein.
12. After incubation, reinstall the cartridge. Flush with loading buffer at 2 ml/min for 5 minutes.

    The ligand is immobilized and you are ready to proceed with the assay. Please refer to the *Performing Unlabeled Perfusion Immunoassays* document also provided.

    If you will not be using the cartridge right away, refer to "Storing the sensor cartridge" on page 6.

Injection method

Plumbing the instrument

Use 0.010-inch I.D. tubing to plumb the system:

Note: Minimize the tubing volume between the column and detector to minimize ligand loss during immobilization.

- INTEGRAL™, BioCad™, BioCad™ RPM™, or BioCad™ Sprint™ systems—Plumb in single-column configuration.
- Other systems—Plumb the instrument to form a gradient and to make repeated 20 µl injections (50 µl sample loop or larger) from the ligand solution. Refer to Figure 4 on page 5.

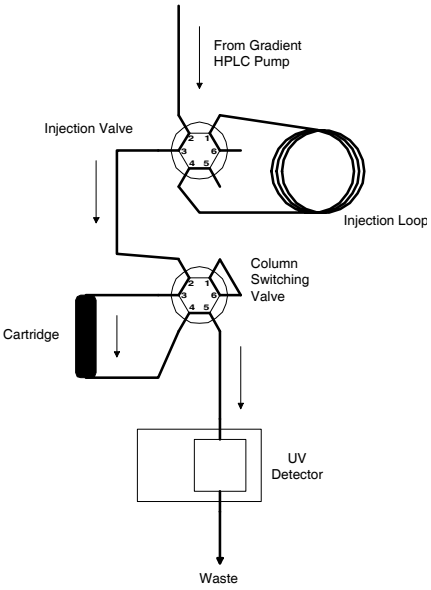

Fig. 4 Injection method plumbing

Preparing the ligand

If your ligand is not an antibody, the salt concentrations of the buffers provided with the kit may not adequately precipitate the ligand on the sensor cartridge.

Before continuing, determine the salt concentration needed. See "Determining salt concentration needed" on page 6.

Keep the total volume of ligand solution as low as possible to minimize the number of 20 µl loading injections needed. With a total ligand volume of 2 ml, it will take 100 injections of 20 µl to fully load the ligand on the cartridge. Injection volumes larger than 20 µl may result in insufficient mixing of ligand with the EP high-salt buffer mobile phase, and therefore less efficient capture of the ligand.

The injection method captures and concentrates ligand on the cartridge.

1. Add one part EP high-salt buffer to two parts of the ligand solution. This yields a solution of 0.5 M salt. Most proteins are soluble at this concentration.
2. If your ligand precipitates, add deionized water to reduce the salt concentration until it just goes back into solution.

Preparing the instrument and cartridge for immobilization

1. Prime gradient line A with EP high-salt buffer.
2. Prime gradient line B with EP low-salt buffer.

Immobilizing the ligand using the injection method

1. Wash and equilibrate the sensor cartridge with at least 5 ml of EP high-salt buffer.
2. Set the flow for gradient line A to 1 ml/min. Make multiple 20 µl injections of the ligand solution. Space the injections 0.5 ml apart. Repeat the injections until the entire sample is loaded onto the sensor cartridge. You should observe small (<0.1 AU) breakthrough peaks with each injection. These peaks may increase in height at the end of the loading if you are approaching the capacity of the cartridge.
3. When all ligand is loaded, run a gradient from 100% A to 100% B over 10 ml, at 1 ml/min. This slowly reduces the salt concentration from 1.5 M to 0.5 M. Do not run the gradient to completion. Closely monitor the UV absorbance at 280 nm. When the ligand begins to elute (absorbance increases 0.05 AU), take the cartridge offline in one of the following ways:
   - Use the column switching valve on the INTEGRAL™, BioCad™, BioCad™ RPM™ , or BioCad™ Sprint™ workstation to put the column in the bypass position.
   - Loosen the inlet to the sensor cartridge.

   Note: Do not turn off the pumps before taking the cartridge offline. Remaining pressure in the system may drive additional liquid through the sensor cartridge.

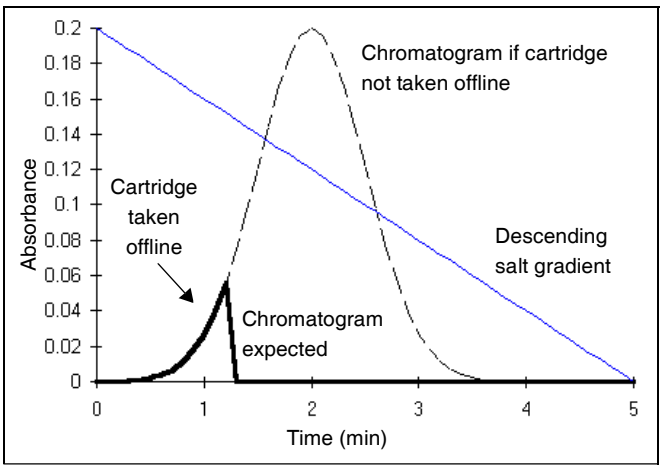

Fig. 5 EP cartridge preparation

4. Remove the cartridge and cap it. Incubate the cartridge for 16 to 24 hours at room temperature.

   Note: Do not refrigerate the cartridge. Salt will precipitate and may damage the POROS™ particles.
5. Flush the system with water to remove salt and protein.
6. After incubation, reinstall the cartridge. Flush with loading buffer at 2 ml/min for 5 minutes.

The ligand is immobilized and you are ready to proceed with the assay. Please refer to the *Performing Unlabeled Perfusion Immunoassays* document also provided.

If you will not be using the cartridge right away, refer to "Storing the sensor cartridge" on page 6 in this document.

Bulk method

Use the bulk method if you are immobilizing ligand on bulk media before packing the cartridge. The following method immobilizes enough ligand on POROS™ EP media for 5 cartridges. Scale the reagents appropriately to make more or fewer cartridges.

Preparing the ligand

Mix ligand solution and EP high-salt buffer in a ratio of 1:1 so that the final salt concentration is 0.75 M. Use a minimum of 1 mg ligand per 50 mg POROS™ EP media.

Keep total volume under 10 ml in a 50 ml tube.

Immobilizing the ligand using the bulk method

1. Note the total volume of ligand/buffer solution prepared above.
2. Add 500 mg (100 mg/cartridge) of POROS™ EP media to the prepared ligand.
3. Mix gently on a rocker or shaker for 5 minutes.

   Note: Do not use a magnetic stir bar and stir plate. POROS™ particles will be ground to fines by the stirrer.
4. Add 1 ml of EP high-salt buffer to the mixture and continue rocking.
5. Calculate the volume of high-salt buffer needed to reach a concentration of 1.1 M using the following formula:

   $$\text{Volume of high-salt buffer to add} = 0.9 \times \text{total volume noted in step 1}$$
6. Repeat step 4 every five minutes until you add the volume calculated above.
7. Mix gently on a rocker or shaker for 16 to 24 hours at room temperature.
8. Filter the media on a 10–20 µm sintered glass funnel.
9. Wash the media in the sintered glass funnel using 50 ml of loading buffer.
10. Wash the media using 50 ml of 1 M NaCl.
11. Wash the media again with 50 ml of loading buffer.

    The ligand is immobilized and you are ready to proceed with the assay. Please refer to the *Performing Unlabeled Perfusion Immunoassays* document also provided.

If you will not be packing the cartridge right away, refer to "Storing the sensor cartridge" on page 6 in this document. Store the bulk media under the same conditions as a packed cartridge.

Determining salt concentration needed

Most protein ligands will be successfully immobilized on the POROS™ EP media surface in the salt concentrations of the buffers provided with the kit. If your ligand is not an antibody, or if you are unsure of the salt levels required to immobilize your ligand, perform the following test.

You need an EP ID sensor cartridge to perform this test.

1. Plumb the instrument as described in "Injection method" on page 5. Put the following buffers on the instrument:
   - Gradient line A: EP high-salt buffer (1.5 M salt)
   - Gradient line B: loading buffer (0 M salt)
2. Prepare the ligand as described in "Injection method" on page 5.
3. Equilibrate the system at 1 ml/min with A: EP high-salt buffer.
4. Inject 20 µl of ligand.
5. Run a gradient from 100% A to 100% B over 10 ml at 1 ml/min.
6. Continue running 100% B for 5 ml. Stop the pump.
7. Observe the resulting chromatogram. You should see:
   - A small breakthrough peak at injection time, showing that the ligand is effectively captured on the EP ID sensor cartridge
   - A broad peak between 1.5 and 0.0 M salt representing elution of ligand

Modify the immobilization method used if you observe the following:

Observation	Recommended Action
Large breakthrough peak at injection time	Salt concentrations used are not sufficient to bring the ligand in contact with the POROS™ EP media. Do not use injection method. If the recycling or bulk methods do not produce a cartridge with satisfactory capacity, consider using a BA ID Sensor Cartridge.
Early elution (between 1.5 and 1.0 M salt) of the ligand in the gradient	**Injection method:** Reduce injection volumes to 10 µl per injection. **Bulk and recycling methods:** Continue salt addition until a final salt concentration of 1.2 M is reached.
Late elution (between 0.5 M and 0.0 M salt) of the ligand in the gradient	**Injection method:** Substitute loading buffer for EP low-salt buffer on gradient line B. **Bulk and recycling methods:** Omit EP high-salt buffer addition steps.

You can use this test cartridge for the following:

- To immobilize the same ligand used in the test with the recycling or injection methods.

  Do not store this cartridge for more than 4 hours before you use it for immobilization. Some hydrolysis of epoxide groups is occurring because the cartridge is now in an aqueous buffer. Some ligand may have covalently immobilized during this test. Do not switch to a new ligand if you intend to use this cartridge for immobilization.
- To test the behavior of other ligands in the salt buffers.

Storing the sensor cartridge

To store the sensor cartridge:

1. Wash the sensor cartridge:
   - Short-term storage—Wash the sensor cartridge with loading buffer prior to storage.
   - Long-term storage—Wash the sensor cartridge with loading buffer containing a bacteriostat such as 0.02 % sodium azide.

   DANGER! CHEMICAL HAZARD. 1% Sodium azide in water is a poison. It may be fatal if inhaled, swallowed, or absorbed through the skin. Exposure may cause nerve and heart damage. Contact with acids liberates toxic gases. DO NOT ADD acids to any liquid wastes containing sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2. Seal the ends using the plugs provided.
3. Store the sensor cartridge at 2 to 8 °C but DO NOT FREEZE.

Accessories, spare parts, and ordering information

Table 2 Spare parts and accessories

Description	Quantity	Cat. No.
ID Self Pack™ Packing Device	1 ea	1-9544-00
Chemistry Trial Kit		
AD, AL, BA, EP, and XL Self Pack™ Media, with reagents and 5 empty cartridges	1 kit	2-3011-00
ID Sensor Cartridge Kits (cartridges with reagents)		
AD ID Sensor Kit	1 kit	2-3006-00
AL ID Sensor Kit	1 kit	2-3010-00
BA ID Sensor Kit	1 kit	2-3005-00
EP ID Sensor Kit	1 kit	2-3009-00
PA ID Sensor Kit	1 kit	2-3001-00
PG ID Sensor Kit	1 kit	2-3002-00
XL ID Sensor Kit	1 kit	2-3004-00
ID Sensor Cartridges (cartridges only)		
AD ID Sensor Cartridge	1 cartridge	2-1007-00
AL ID Sensor Cartridge	1 cartridge	2-1010-00
BA ID Sensor Cartridge	1 cartridge	2-1005-00
EP ID Sensor Cartridge	1 cartridge	2-1009-00
PA ID Sensor Cartridge	1 cartridge	2-1001-00
PG ID Sensor Cartridge	1 cartridge	2-1002-00
XL ID Sensor Cartridge	1 cartridge	2-1004-00
Self Pack™ Media		
AD (Adsorbed)	500 mg	2-3114-00
AL (Aldehyde)	500 mg	2-3115-00
BA (Streptavidin)	1 ml	2-3111-00
EP (Epoxide)	500 mg	2-3116-00
PA (Protein A)	1 ml	2-3117-00
PG (Protein G)	1 ml	2-3118-00
XL (Cross-linked Protein G)	1 ml	2-3119-00
Reagents		
Loading Buffer	1 pack	2-2101-00
Elution Buffer	1 pack	2-2102-00
AD ID Reagent Pack	1 set	2-3120-00
AL ID Reagent Pack	1 set	2-3121-00
EP ID Reagent Pack	1 set	2-3122-00
XL ID Reagent Pack	1 set	2-3124-00
Empty Cartridges		
2.1 mmD/30 mmL PEEK	Pkg of 5	2-3008-05
2.1 mmD/30 mmL PEEK	Pkg of 10	2-3008-10
2.1 mmD/30 mmL PEEK	Pkg of 20	2-3008-20
Accessories, Packing Device		
Fitting Adaptor Kit	1 ea	1-9532-00
O-Ring	1 ea	1-9108-00
Teflon Washer	1 ea	1-9147-00
Accessories, Cartridge		
Frits, 2.1 mm PEEK	Pkg of 5	1-9124-05
E-Z Grip™ Fittings (SS)	Pkg of 5	5-1011-05
Fitting Adapter Kit	1 kit	1 -9532-00

Support

For service and technical support, go to **thermofisher.com/poros** or call toll-free in US: 1.800.831.6844.

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to **thermofisher.com/support**, or contact you local Thermo Fisher Scientific representative.

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Revision	Date	Description
B	27 March 2017	Baseline for this revision history.

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