AD ImmunoDetection™ Sensor Cartridge 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**.

Your new ID Sensor Cartridge is unique

Thermo Scientific™ ImmunoDetection™™ (ID) Sensor Cartridges for Perfusion Immunoassay™ overcome many of the limitations of conventional immunoassays performed in microtiter plates.

A Perfusion Immunoassay™ is a flow-through, solid-phase immunoassay that is:

- **Fast**— They eliminate the manual, labor-intensive steps performed in conventional immunoassays. Rapid, intimate contact between analyte and ligand promotes rapid capture and eliminates long incubation steps.
- **Precise and Reproducible**— You run samples and standards on the same flow-through ID Sensor Cartridge (the solid support on which ligands are immobilized or proteins are bound).
- **Automated**— Novel ID technology eliminates manual operations.

ID technology is an extension of Perfusion Chromatography™ technology, combining flow-through POROS™ chromatographic particles with antibody, antigen, or other binding molecules.

Please read "Understanding Perfusion Immunoassay™ and ImmunoDetection™ technology" on page 5 for more information.

Product description

The AD ImmunoDetection™ (ID) Sensor Cartridge is a general-purpose assay device for measurement of antigens and/or antibodies.

Note: Avoid using the AD sensor cartridge with detergents and high concentrations of hydrophobic solutes. These compounds can cause significant desorption of many ligands, including antibodies. If you must use organic solvents, consider using a different ID sensor cartridge chemistry.

For information on other sensor cartridge chemistries available, see "Selecting an ID Sensor Cartridge" on page 7.

Ligand immobilization

The AD (Adsorptive) sensor cartridge immobilizes ligand by simple adsorption. Most antibodies and many antigens can be efficiently and rapidly immobilized by non-covalent adsorption onto the AD sensor cartridge. This adsorption is essentially irreversible and yields a stable solid-phase separation that can be reused.

Because of its broad applicability, simplicity, and minimal loss of activity of the adsorbed material, simple adsorption is often the first method chosen for development of immunoassays. Any molecule that adsorbs with sufficient activity to other polymer surfaces (such as microtiter plate wells or latex) will adsorb to the AD sensor cartridge.

Materials provided

The AD ImmunoDetection™ Sensor Cartridge kit includes:

- AD ImmunoDetection™ Sensor Cartridge (2.1 mmD × 30 mmL)
- Loading buffer, 1 pack (10 mM phosphate, 0.15 M NaCl, pH 7.2)
- Elution buffer, 1 pack (0.15 M NaCl, pH 2 to 3)
- Blocking solution, 1 vial [30 mg bovine serum albumin (BSA)]

Materials required but not provided

- INTEGRAL™ Micro-Analytical Workstation, BioCad™ Workstation, 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 5 ml/min.
- HPLC sample loop twice the volume of the largest injection
- 12 M HCl

Note: A fitting adaptor kit is available from **thermofisher.com** to connect the ID sensor cartridge to metric FPLC™ systems and to 1/4-28 thread fitting systems.

Overview of Perfusion Immunoassay™ and ImmunoDetection™ technology

Steps of a Perfusion Immunoassay™

The AD immunoassay involves the following steps:

- **Immobilization**—Ligand (antibody, antigen, or other binding protein) is immobilized onto a sensor cartridge containing POROS™ AD media. Ligand is adsorbed onto the entire surface and pores of the POROS™ flow-through media. Remaining surface area is blocked with a protein such as BSA.
- **Binding**—Sample is flowed through the sensor cartridge where the target analyte specifically binds to the ligand immobilized on the sensor cartridge.

  Because of the high concentration of the immobilized ligand and the rapid transport of analyte throughout the flow-through POROS™ media, the binding of the target analyte is nearly instantaneous.
- **Wash**—Non-target components of the sample are easily washed from the sensor cartridge.

- **Elution**—Bound analyte is released by dissociating the antigen:antibody complex.
- **Detection**—Eluted target analyte is detected using UV detection or other methods.
- **Regeneration**—Sensor cartridge is regenerated with sample loading buffer for reuse.

Figure 1 on page 2 illustrates antibody immobilization on the adsorptive AD sensor cartridge surface.

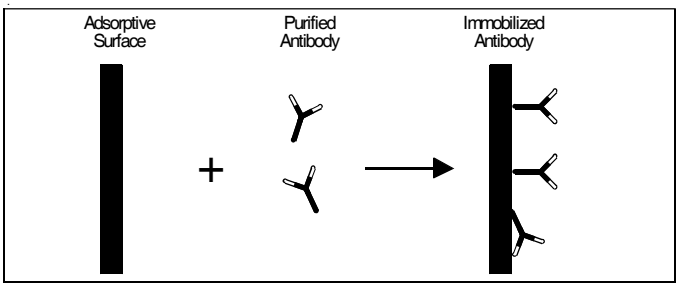

Fig. 1 Antibody immobilization on the AD Sensor Cartridge

Direct detection assays

Direct detection assays take advantage of the concentrating capability of chromatography. Analyte in the sample is captured and concentrated in the sensor cartridge.

Bound analyte is then eluted from the sensor cartridge by dissociating the antigen:antibody complex and detecting the eluted material directly, usually with an optical absorbance detector.

Figure 2 on page 2 illustrates an antibody-based direct detection assay.

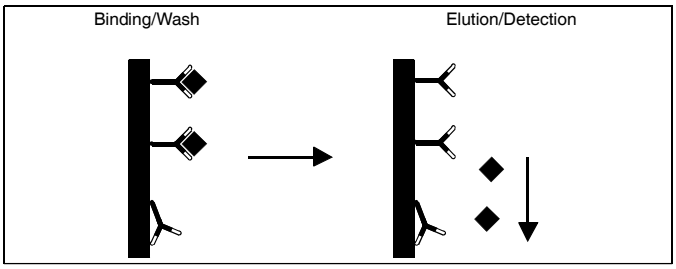

Fig. 2 Direct detection assay

Other assay formats

Ultrasensitive labeled immunoassays (for example, enzyme immunoassays, fluorescent assays, and chemiluminescent assays) can also be performed on ImmunoDetection™ sensor cartridges.

For details on these techniques, contact Technical Services (see "Support" on page 7).

Selecting and preparing a ligand

All ligands (antigens, antibodies, or receptors) and contaminating proteins bind to the AD sensor cartridge. Therefore, you need as pure a ligand as possible. A total ligand mass of 10 nmol is suggested. Antibodies can be purified on Protein G (see "Selecting an ID Sensor Cartridge" on page 7 for information about ID sensor cartridges containing Protein G).

The qualities of your ligand influence assay performance and sensor cartridge lifetime. Each antibody or receptor is unique, and we can offer only general guidance on its use. Antibodies must be of high enough affinity to capture antigens out of solution as they flow past. Also, antigen:antibody complexes must be reversible under conditions that do not irreversibly denature the antibody. While most antibody reagents satisfy these conditions, not all antibodies can be guaranteed to be satisfactory.

Antigen: antibody interactions

The high surface area of POROS™ flow-through media and the short diffusional distances permit extremely rapid immunoassays. In a flow-through Perfusion Immunoassay™, three factors can potentially influence assay sensitivity and speed (reaction time necessary to achieve a given level of sensitivity):

- Concentration of ligand
- Flow rate
- Affinity constant of ligand

The figures below qualitatively illustrate the effects of varying these parameters.

As the immobilized ligand concentration is increased, the sensitivity, speed, and dynamic range of the assay can be increased (Figure 3 on page 2).

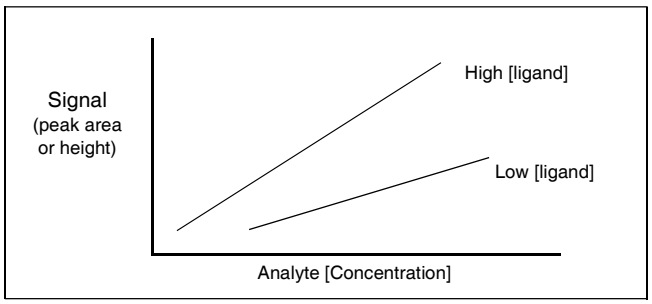

Fig. 3 Effect of varying concentration of immobilized ligand

High flow rates minimize the contact time for binding and may reduce assay sensitivity (Figure 4 on page 2). For high-affinity ligands immobilized at high concentrations, flow rate effects are minimal.

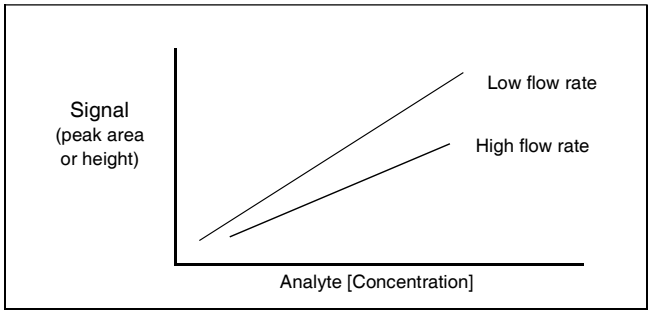

Fig. 4 Effect of varying flow rate

Higher ligand affinity constants result in more sensitive assays (Figure 5 on page 2).

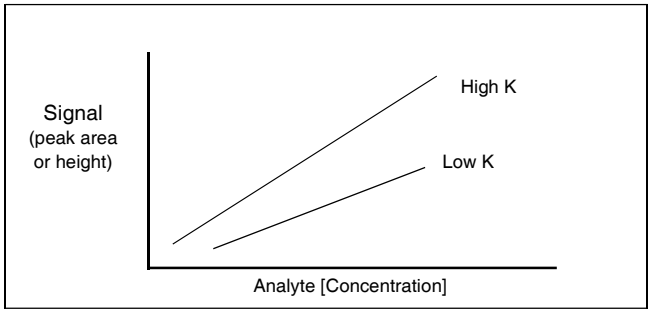

Fig. 5 Effect of different ligand affinity constants (K)

Assay considerations

Detection range

In general, dynamic range and assay sensitivity are greater if more ligand is immobilized onto the sensor cartridge. The maximum antibody ligand immobilizing capacity of the sensor cartridge is approximately 12.5 nmol. Higher quantities of ligand allow you to run at higher flow rates, provide linear assay responses over a larger dynamic range, and provide maximum assay flexibility. Lower

quantities of immobilized ligand can still yield good assay results. However, to estimate how much ligand to immobilize onto the sensor cartridge and what volume of sample to analyze, you must consider the concentration range of the analyte to be detected and its UV detection sensitivity.

With UV detection, lower detection limits range from 0.1 to 1 pmol, varying with analyte mass, extinction coefficient, sample matrix, and wavelength used for detection. Wavelengths between 210 and 220 nmol yield maximum response. If UV detection is not sensitive enough for your application, use some other detection method such as enzyme amplification, fluorescence, or chemiluminescent labels.

The upper end of the dynamic range can be as high as 5 nmol. Analyte binding capacity can be compromised by low ligand purity, poor coupling efficiency and low ligand affinity constant.

You can increase the upper quantitative range of some assays by using higher flow rates. Conversely, you can sometimes improve assay sensitivity by using slower flow rates.

Mass of eluted peak

Because the flow-through sensor cartridge acts as a high-capacity, highly efficient concentrator of analyte, detection can be based on the mass of the eluted peak instead of on the concentration of the analyte in solution. Thus the sample volume required for the direct detection assay is a function of analyte concentration in the sample and the detection limit needed.

For example, in very dilute samples, a larger volume can be used to obtain the required minimum detectable mass. However, the validity of this variable volume approach must be established for each assay system. Factors such as ligand affinity, sensor cartridge capacity, and flow rate can limit the valid application of the variable-volume approach.

To determine validity experimentally, compare the mass dose response curves of a fixed-volume variable concentration assay to those of a variable-volume fixed concentration assay. The extent of overlap or linearity of these two sets of data indicates the range of sample volumes you can use. For most applications, it is safest to run a fixed-volume standard curve for each of the sample volumes you test.

Nonspecific binding

The sensitivity of the UV direct detection assay can be affected by the mass of UV-absorbing material that nonspecifically binds and subsequently elutes.

This nonspecific binding depends on the matrix composition of the sample. You can determine the nonspecific binding interference for various sample types and volumes by measuring the UV absorbance of eluted samples that contain no analyte (see “Evaluating nonspecific binding“ on page 4).

The change from the loading buffer to the elution buffer can also produce nonspecific background signals, resulting from refractive index disturbances.

Although the sensor cartridges normally yield minimal nonspecific binding, it is often possible to further minimize nonspecific binding by using buffer additives (see “Reducing nonspecific binding“ on page 4).

Preparing for the assay

The steps you must take to prepare the different elements of your assay are described in the following sections:

- Connecting the Sensor Cartridge
- Preparing the Loading Buffer
- Preparing the Elution Buffer
- Preparing the Blocking Agent
- Immobilizing the Ligand
- Preparing the Sensor Cartridge for Use

Using different loading or elution buffers

If you do not use the loading or elution buffers supplied with the kit, keep these guidelines in mind as you select buffers:

- Do not use isothiocyanate solutions as elution buffers. They may react with stainless steel components of the HPLC system.
- With certain ligands immobilized on the AD sensor cartridge, antigen can elute as two peaks.

  To eliminate the double peak during adsorption, add approximately 400 µg of BSA or normal IgG to the antibody.

  To include the area of both peaks in the quantitation after elution, set a wide peak width for integration.
- The loading and elution buffers supplied with the kit are developed to produce minimal baseline changes due to refractive index disturbances when switching from loading buffer to elution buffer. If you use a different elution system, determine if there is any interference from refractive index changes and adjust the components of loading and elution buffers to eliminate the refractive index changes, or allow for refractive index changes in your analysis of the data.
- If the eluted peak area decreases significantly from run to run, the elution buffer may be denaturing the ligand. Try other elution schemes or consider other methods of immobilizing the ligand. Not all elution buffers work with all antibodies. A good first choice is 12 mM HCl. Alternatives include 50% ethylene glycol in loading buffer, and 10 mM ethanolamine, pH 11.5. Other approaches can use other low-pH agents, high-pH agents, or chaotropes.

IMPORTANT! Do not use detergents or solvents.

Connecting the sensor cartridge

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. Overtightening can strip the threads of the ID sensor cartridge.

To connect a sensor cartridge:

1. Remove the end plugs from the sensor cartridge. Save the plugs for future storage of the cartridge.
2. Slip a stainless steel 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 1/16 inch of tubing extends from the ferrule.

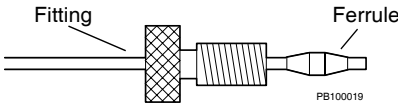

Fig. 6 Connecting E-Z Grip™ fittings

4. Bottom the tubing in the end fitting of the cartridge. Fingertighten the compression fitting. Do not overtighten.

Preparing the loading buffer

To prepare the loading buffer supplied with the kit:

1. Add the contents of the loading buffer package to 1L of deionized water.
2. Stir until buffer crystals dissolve.
3. Check pH and adjust to 7.2 if necessary (use HCl or NaOH).
4. Filter the loading buffer solution using 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.

Preparing the elution buffer

To prepare the elution buffer supplied with the kit:

IMPORTANT! Do not leave the sensor cartridge in elution buffer. Elution buffer can degrade the sensor cartridge and shorten its lifetime.

1. Add the contents of the elution buffer package to 1 L of deionized water.
2. Stir until buffer crystals dissolve.
3. Add 1 ml of 12 M HCl (37% by weight) for a final elution buffer concentration of 12 mM HCl.
4. Check that the pH is between 2 and 3. Adjust if necessary using 12 M HCl.
5. Filter the elution buffer solution using 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.

Preparing the blocking agent

1. Reconstitute the contents of the blocking agent vial with 3 ml loading buffer.
2. Filter the blocking solution using a 0.22-µm filter before use.

Note: Store unused blocking agent at 2 to 8 °C.

Immobilizing the ligand

To immobilize the ligand:

1. Choose the appropriate sample loop size.
2. Wash the sample loop with loading buffer.
3. Equilibrate the AD sensor cartridge with loading buffer at 3 ml/min for 3 minutes.
4. Load the sample loop with ligand solution and inject it onto the sensor cartridge at 0.5 ml/min. Use repeat injections for large volumes.
5. Wash the loop with appropriate volume of loading buffer.
6. Wash the sensor cartridge with loading buffer at 3 ml/min for 1 minute.
7. Load the sample loop with blocking solution and inject it onto the sensor cartridge at 0.5 ml/min.
8. Inject at least 5 mg of blocking protein.
9. Flush the sensor cartridge with loading buffer at 3 ml/min for 5 minutes.

IMPORTANT! Do not leave the sensor cartridge in elution buffer. Elution buffer can degrade the sensor cartridge and shorten its lifetime.

Preparing the sensor cartridge for use

Before you run a sample on the sensor cartridge, you must:

- Condition a new sensor cartridge
- Verify that the sensor cartridge binds the analyte of interest and that the analyte is recovered in the elution buffer
- Evaluate and reduce nonspecific binding

Conditioning a new sensor cartridge

To condition a new sensor cartridge:

1. Select a flow rate of 0.1 to 5 ml/min.

   Lower flow rates give slightly greater sensitivity at the expense of assay speed. Choose a flow rate consistent with your needs for sensitivity and sample throughput, and that does not exceed the high-pressure limit for your system or the sensor cartridge.
2. Run 2 ml of loading buffer through the sensor cartridge.
3. Run 2 ml of elution buffer through the sensor cartridge.
4. Run 2 ml of loading buffer through the sensor cartridge.
5. Repeat steps 3 and 4 two or three times, until you establish a stable baseline.

IMPORTANT! Do not leave the sensor cartridge in elution buffer. Elution buffer can degrade the sensor cartridge and shorten its lifetime.

Verifying that the sensor cartridge works for your assay

To verify that the sensor cartridge works for your purposes:

1. Set a flow rate of 0.1 to 5 ml/min, then select the absorbance detection wavelength (usually 280 or 220 nm. You will see larger signals at 220 nm, but will also see increased backgrounds).

   IMPORTANT! You can increase the flow rate to speed up the assay. However, sensitivity may decrease at higher flow rates. Do not exceed a sensor cartridge pressure of 2500 psi.
2. Inject a sample of the target molecule onto the sensor cartridge.

   Use a sample loop of approximately twice the injection volume. Do not inject more than one half the loop volume at one time.
3. Wash the sensor cartridge with loading buffer until the UV absorbance returns to baseline.
4. Switch to the elution buffer.
5. Continue flowing elution buffer until the analyte peak of interest completely elutes.
6. Switch back to the loading buffer to regenerate the sensor cartridge.
7. Repeat the above procedure until a consistent eluted analyte peak is obtained.

Evaluating nonspecific binding

To find out if your loading buffer or sample matrix is producing nonspecific binding:

1. Wash and equilibrate the sensor cartridge with at least 2 ml of filtered loading buffer.
2. Inject an analyte-free sample at 1 ml/min.
3. Elute with elution buffer.
4. Observe the UV detector signal to determine if any matrix or analyte binds and elutes from the sensor cartridge in the absence of analyte.
5. Reequilibrate the sensor cartridge in loading buffer.

Reducing nonspecific binding

If nonspecific binding interferes with the sensitivity of your immunoassay, you can reduce nonspecific binding by adding the following to your sample:

- 0.1% of a carrier protein such as BSA
- 0.5 M NaCl

IMPORTANT! Do not use detergents. If you add salt to your loading buffer, make sure to add to elution buffer also.

Performing the assay

This section describes how to perform your assay. It contains these sections:

- Preparing a Sensor Cartridge After Long-Term Storage
- Generating a Standard Curve
- Running the Sample

Preparing a sensor cartridge after long-term storage

Recondition the sensor cartridge after long-term storage (more than one week). Follow the procedure in "Conditioning a new sensor cartridge" on page 4.

Generating a standard curve

To generate a standard curve:

1. Make standards up in the same matrix as the samples.
2. Run a buffer blank or sample devoid of the target molecule to serve as a baseline blank or zero standard.
3. Set a flow rate of 0.1 to 5 ml/min and select absorbance detection wavelength (usually 280 or 220 nm—you will see larger signals at 220 nm but may also see increased backgrounds).
4. Set the data acquisition rate appropriate for the selected flow rate. Higher flow rates require a higher data acquisition rate. At the highest flow rate, set the data acquisition rate to 10 points/sec (10 Hz).

5. Inject a standard.

   To cover the range of interest for the assay, you can do one of the following:

   - Use the same volume and different concentrations of the standard. This is the recommended method.
   - Use different injection volumes (typical injection volumes range from 1 to 1000 µl).

6. Wash the sensor cartridge with loading buffer until the baseline is reached, then switch to the elution buffer.
7. Continue flowing elution buffer until the analyte peak of interest completely elutes.
8. Run each standard in duplicate or triplicate.
9. Integrate the eluted analyte peak areas.
10. Plot the standard eluted analyte peak area as a function of mass of analyte standard.

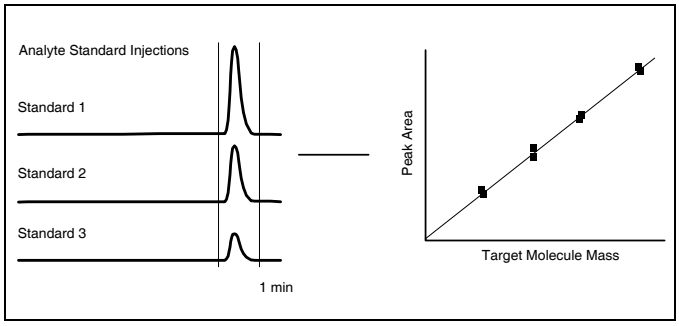

Fig. 7 Plotting the standard curve

Running the sample

Note: Sample concentration must be within the standard curve range. Dilute the sample if the concentration is higher than the maximum detection limit for the assay.

Run samples under the same conditions used to generate the standard curve.

To perform the assay:

1. Set a flow rate and select the absorbance detection wavelength. Use the same settings you used to generate the standard curve.
2. Inject the sample.

   Use a sample loop of approximately twice the injection volume. Do not inject more than one half the loop volume at one time.
3. Wash the sensor cartridge with loading buffer until the baseline is reached. Switch to the elution buffer.
4. Continue flowing elution buffer until the analyte peak of interest completely elutes.
5. Reequilibrate the sensor cartridge in loading buffer after the last run.
6. Integrate the sample eluted analyte peak area.
7. Interpolate the analyte mass in the sample from the standard curve (Figure 8 on page 5).

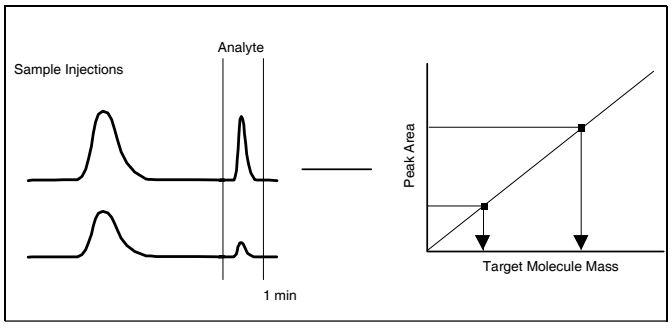

Fig. 8 Determining sample mass

8. To calculate the sample analyte concentration, use this formula:

$$\text{concentration} = \text{mass from standard curve} \div \text{sample volume} \times \text{dilution factor}$$

Regenerating and storing the sensor cartridge

To avoid denaturation of the immobilized ligand, quickly restore the sensor cartridge to neutral conditions after elution of the bound fraction. Conditions depend on your immunoassay.

If sensor cartridge backpressure exceeds 2000 psi, backflush with alternating cycles of loading and elution buffers. Do not exceed a sensor cartridge pressure limit of 2500 psi.

To store the sensor cartridge:

- Store the sensor cartridge at 2 to 8 °C but DO NOT FREEZE.
- Seal the ends using the plugs provided.

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.

CAUTION! Sodium azide is toxic. Follow precautions and decontamination procedures recommended by the National Institute for Occupational Safety and Health.

Understanding Perfusion Immunoassay™ and ImmunoDetection™ technology

The following novel technology and instrumentation dramatically improve the efficiency of immunoassay protocols:

- ImmunoDetection™ technology
- Perfusion Immunoassay™ technology
- INTEGRAL™ Micro-Analytical Workstation

ImmunoDetection™ technology

ImmunoDetection™ technology is an extension of Perfusion Chromatography™ technology.

An ID sensor cartridge is packed with POROS™ flow-through beads, and ligand is immobilized on the POROS™ beads.

The flow-through bead is an ideal environment for immunoassay. The bead provides a large, readily accessible surface area, equivalent to thousands of interconnected microtiter wells. Target molecules are exposed to an excess of ligand immobilized within the pores of the beads. Excess ligand promotes rapid target capture.

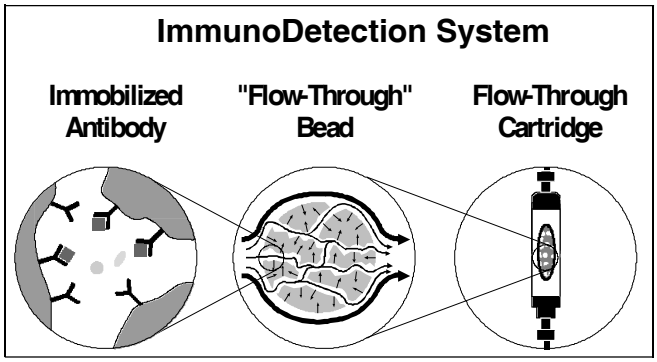

Fig. 9 ImmunoDetection™ system

The convective transport property of POROS™ media:

- Rapidly transports antigen to immobilized antibody within the pores of the perfusive media
- Rapidly and efficiently captures and concentrates antigen

POROS™ media pore structure—large throughpores and smaller diffusive pores—carries analyte to particle interiors far more rapidly than other conventional chromatographic media. Flow-through POROS™ beads, therefore, create a large amount of readily accessible surface area (1 m^2 /cartridge). Rapid, intimate contact between analyte and ligand promotes rapid capture, eliminating long incubation steps.

Advantages of this flow-through technology include:

- **Assays are not limited by sample volume**—Assays occur in a flow-through cartridge, instead of a in microtiter well
- **Background interference is minimized**—Non-target components are easily washed and removed
- **Cartridge can be reused hundreds of times**—Reduces cost and improves precision of the assay

ID Sensor Cartridge Kits are available with various chemistries for attachment of desired ligands, including pre-immobilized Protein A and Protein G, chemistry for covalent attachment of biomolecules, and an avidin-activated surface for immobilization of biotinylated ligands (see "Selecting an ID Sensor Cartridge" on page 7).

Perfusion Immunoassay™ technology

Perfusion immunoassays, also referred to as flow-through immunoassays, are solid-phase immunoassays performed using ImmunoDetection™ (ID) technology.

Perfusion immunoassay run in a chromatographic medium that allows:

- Extremely rapid fluid flow
- Highly efficient contact between antigen and antibody
- Specificity of antigen:antibody interactions

In a Perfusion Immunoassay™, sample antigen or antigen:antibody complexes in a flowing buffer stream are swept into the ImmunoDetection™ sensor cartridge, where the antigen/target analyte is captured by interaction with specific immobilized antibody.

The amount of immobilized antibody or other capture material generally far exceeds the amount of antigen. Because liquid passes through this bed of immobilized antibody, antigen can be captured from a sample volume many times larger than the volume of the assay chamber.

Long incubation steps are unnecessary. Cartridge residence times of seconds are sufficient for complete analyte capture. These residence times allow almost instantaneous antigen capture, as well as antigen concentration from dilute sample in the assay chamber prior to the assay.

The INTEGRAL™ Micro-Analytical Workstation

The INTEGRAL™ Micro-Analytical Workstation, an advanced workstation that optimizes ID cartridge functionality and allows you to run a Perfusion Immunoassay™ in tandem with other chromatographic separation and analytical technologies.

The INTEGRAL™ Workstation is a self-contained, bench-mounted Perfusion Immunoassay™/micro-analytical liquid chromatography workstation containing the software, plumbing, valving, and instrumentation to automate a wide range of flow-through assays.

Proprietary ImmunoDetection™ and Perfusion Chromatography™ technology are the key to the INTEGRAL™ Workstation performance.

The INTEGRAL™ software introduces a computer-aided approach to the development of high-sensitivity and high-throughput immunoassays to characterize and analyze biomolecules.

For more information on the INTEGRAL™ Micro-Analytical Workstation, contact us (see "Support" on page 7).

Note: ID cartridges can also be plumbed to any LC/HPLC instrument that can run two solvents and has a data system capable of obtaining peak areas.

Accessories, spare parts, and ordering information

Table 1 on page 6 lists the accessories available from **thermofisher.com**:

Table 1 Spare parts and accessories

Description		Quantity	Cat. No.
Kits (includes sensor cartridge and necessary reagents)			
AD ID Sensor Cartridge Kit		1 kit	2-3006-00
BA ID Sensor Cartridge Kit		1 kit	2-3005-00
CO ID Sensor Cartridge Kit		1 kit	2-3003-00
PA ID Sensor Cartridge Kit		1 kit	2-3001-00
PG ID Sensor Cartridge Kit		1 kit	2-3002-00
PG/AD ID Sensor Cartridge Kit		1 kit	2-3007-00
XL ID Sensor Cartridge Kit		1 kit	2-3004-00
Replacement Cartridges			
AD ID Sensor Cartridge		1 cartridge	2-1007-00
BA ID Sensor Cartridge		1 cartridge	2-1005-00
CO ID Sensor Cartridge		1 cartridge	2-1003-00
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
Replacement Reagents			
Blocking solution		1 vial	2-2102-00
Coupling buffer		1 pack	2-2108-00
Cross-linking solutions	Triethanolamine	1 vial	2-2105-00
	Dimethyl pimelimidate (DMP)	1 vial	2-2106-00
Elution buffer		1 pack	2-2104-00
Loading buffer		1 pack	2-2101-00
Quenching solution		1 vial	2-2107-00
Salt precipitation buffer		1 pack	2-2103-00
Frits, PEEK		Package of 5	1-9124-05
E-Z Grip™ Fittings (SS)		Package of 5	5-1011-05
Fitting Adaptor Kit[1]		1 kit	1 -9532-00

[1] The Fitting Adaptor Kit lets you connect POROS™ columns to M-6 (FPLC) and 1/4-28 low-pressure fitting systems. The kit includes two 10-32 fittings, two low-pressure ferrules, two M-6 nuts, two 1/4-28 nuts, and 1/16-inch O.D. PEEK tubing. The kit is included with all F-and P-Series columns.

Selecting an ID Sensor Cartridge

Use Table 2 on page 7 to determine the ID Sensor Cartridge appropriate for your immunoassay. If you have any questions when selecting the ligand to immobilize, please contact us (see "Support" on page 7).

Table 2 Selecting ID Sensor Cartridge kits

Application	Sensor Cartridge	Immobilization Method	Immobilization Target	Comments
General antibody or antigen immobilization	AD	Adsorption	All antibodies and most antigens	• Requires blocking agent. • Avoid using with detergents and organics, which may cause desorption of ligand. • Nonspecific immobilization of protein requires pure ligand to achieve high binding capacity.
Immobilization of impure IgG on AD cartridge	PG/AD	Adsorption	Immunoglobulin G	• Relatively impure IgG from anti-sera, ascites, or cell culture media can be used. • Requires blocking agent. • Avoid using with detergents and organics, which may cause desorption of ligand.
Immobilization of any IgG species or subclass that binds to Protein G	XL	Protein G binding to Fc regions of immunoglobulins followed by covalent cross linking	Immunoglobulin G	• Orients antibody with Fab regions exposed giving high activity. • Does not require purified IgG for immobilization, because the XL sensor cartridge selectively captures IgGs and does not capture non-IgGs. **Note:** If you want to immobilize a specific antibody, start with an affinity-purified antibody. • Sample matrixes containing immunoglobulin may interfere in detection of the intended antigen by giving higher backgrounds.
Direct covalent immobilization of antibody or antigen that can withstand immobilization at high pH	CO	Covalent cross-linking of epoxide surface to various functional groups of material to be immobilized	Antibody or antigen, through amines, sulfhydryls, and hydroxyls	• May require excess antibody to maximize capacity. • Immobilization at pH 9 may denature some ligands.
Immobilization of biotinylated antibody or antigen	BA	Biotinylated ligand binding to streptavidin surface	Biotinylated antibody or antigen	Strong avidin-biotin affinity results in strong noncovalent interaction that is not disrupted under most elution conditions.
Application	**Sensor Cartridge**	**Immobilized Ligand Supplied on Cartridge**	**Intended Use**	**Comments**
Antibody quantitation	PA	Protein A activated surface	Detection of various species and subclasses of IgG	Binds immunoglobulin G of different species and subclasses with varying affinity. Does not bind mouse IgG_1, human IgG_3, or rat and goat IgGs.
Antibody quantitation	PG	Protein G activated surface	Detection of various species and subclasses of IgG	Binds a wider range of species and subclasses than Protein A, including mouse IgG_1.

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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision	Date	Description
C	10 January 2017	Baseline for this revision history.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288