



Prisma ELISA™ kit

Instructions for Use

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1 Introduction

Product code

29707299

Important user information

Read these instructions carefully before using the product.

Intended use

The product is intended for research or manufacturing use only, and must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety precautions

All chemicals should be considered as potentially hazardous. Avoid contact with skin or eyes, in case of contact wash immediately with water. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Only trained users must use this product in accordance with the principles of good laboratory practice.

See material safety data sheets and/or safety statements for specific advice.



CAUTION

Contains egg derivatives. Use personal protective equipment to prevent contact with skin or inhaling powders. Some components might produce an allergic reaction in susceptible individuals.

Storage

Store all kit components at 2°C to 8°C upon arrival. Aliquot the reconstituted protein standard and store at -20°C.

Expiration

The expiration date is shown on the package and is at least four weeks from the delivery date.

2 Background

Description of the product

The PrismA ELISA™ kit (29707299) is a sandwich ELISA kit designed to detect residual ligand contamination in samples from process development, manufacturing, and batch release of biopharmaceuticals purified using MabSelect PrismA™ chromatography resin and Fibro PrismA chromatography adsorbers. The kit uses purified antibodies generated specifically against MabSelect PrismA ligand.

Compatibility with other ProteinA derived ligands has not been tested and can not be guaranteed.

Assay description

Residual ligands of MabSelect PrismA in process-derived samples are dissociated from drug products, then captured in a microplate. The plate is pre-coated with a purified, polyclonal, PrismA specific antibody.

The captured proteins are detected using an HRP-conjugated, polyclonal, anti-PrismA antibody. The addition of TMB results in a chromogenic change that can be measured photometrically at 450 nm with reference wavelength at 630–650 nm. The optical density (O.D.) in the wells is directly proportional to the concentration of MabSelect PrismA ligand and correlates to the amount of ligand present in the original sample. The MabSelect PrismA ligand concentration can be calculated based on the calibration curve generated with the provided standard.

The levels of detection and quantification of ligand in samples derived from PrismA affinity chromatography are process-dependent and might be limited.

Assay performance characteristics

The complete validation package is available in the accompanying data file available from the **Documents** tab on the product page. Visit <http://cytiva.com> to access the data file.

The table below shows the general kit specifications.

Note: For specific lot parameters refer to the Certificate of Analysis (CoA). Visit <http://cytiva.com/certificates> and search by lot number.

Parameter	Specification
Sensitivity, lower limit of detection (LLD)	10 pg/mL in 1 mg/mL IgG
Sensitivity, lower limit of quantification (LLQ)	10 pg/mL in 1 mg/mL IgG
Precision, intra- and inter-assay CV%	< 20%
Range of the standard curve	0.018 to 4.5 ng/mL
Recovery of each standard	80% to 120%

3 Materials

Kit components

Component	Size
Prisma ELISA kit 96 well strip-plate	1 plate
Prisma ELISA kit detection antibody (100×)	140 µL/vial
Prisma ELISA kit detection antibody diluent (1×)	15 mL/bottle
Prisma ELISA kit assay buffer (1×)	10 mL/bottle
Prisma ELISA kit wash buffer (10×)	25 mL/bottle
Prisma ELISA kit TMB (1×)	15 mL/bottle
Prisma ELISA kit stop buffer	15 mL/bottle
Prisma ELISA kit dissociation buffer (1×)	30 mL/bottle
Prisma ELISA kit plate sealer	1 sheet
Prisma ELISA kit standard	112.5 ng/vial

Reagents and materials required but not provided

- Microcentrifuge tubes (1.5 mL)
- Single-channel pipettes (2–20 µL, 10–100 µL, and 100–1000 µL) and tips
- Multichannel pipette (50–300 µL) and tips
- Spectrophotometer (microplate reader) capable of reading 96-well plates at 450 nm with a reference filter at 630–650 nm
- Reagent reservoirs
- Plate shaker
- Vortex mixer
- Stir plate and magnetic stir bar
- Absorbent paper
- Timer
- Polypropylene centrifuge tubes (15 mL)
- Deionized water
- Disposable gloves
- Graduated cylinder

- Deep-well (600 µL) microplate
- (optional) Automatic plate washer and PBS + 0.05% (v/v) Tween-20

4 Assay reagents preparation

Introduction

Prepare the wash buffer and the assay standards before beginning the assay. Prepare the detection antibody working solution just before adding it to the plate

Prepare the wash buffer

Follow the steps below to prepare the wash buffer (1×).

Step	Action
1	Add 25 mL of Prisma ELISA kit wash buffer (10×) to 225 mL of deionized water.
2	Mix for at least 10 minutes using a magnetic stir bar.
Tip:	<i>If using an automatic plate washer, and the volume of wash buffer (1×) is not sufficient, a solution of PBS + 0.05% (v/v) Tween-20 can be used. Larger volumes of wash buffer can also be purchased from Cytiva (product code 28995084).</i>
Note:	<i>The wash buffer (1×) can be stored at room temperature (15°C to 25°C) for up to 2 weeks. After 2 weeks, the wash buffer (1×) should be discarded.</i>

Prepare the assay standards

A standard curve must be generated for every assay. The stock of the standard, Prisma ELISA kit standard (225 ng/mL), provided in the kit is enough to run several sets of assay standards at the described dilutions and to run spike control tests to monitor matrix specific effects.

To prepare the assay standard stock solution, reconstitute the Prisma ELISA kit standard with 500 µL of deionized water to obtain a final concentration of 225 ng/mL (stock). Mix the vial with a vortex mixer.

Tip: *The Prisma ELISA kit standard is packaged under vacuum. To prevent loss of standard, remove the rubber stopper slowly when opening the vial.*

Tip: *If the Prisma ELISA kit standard is used in subsequent assays, dispense the stock in small aliquots and store it at -20°C for up to twelve months.*

To prepare a serial dilution of the assay standards, see [Prepare the samples, on page 10](#).

Note: *For most applications, it is not necessary to acquire additional Prisma ligand than provided in the assay.*

For users requiring more ligand, the MabSelect PrismaA Ligand Restricted License (29236787) is available separately. This product forms dimers in solution which may impact performance of the ELISA and therefore must be modified to be representative of leached PrismaA ligand and be compatible with the assay. For more information contact your local Cytiva representative.

Prepare the detection antibody working solution

Prepare the detection antibody working solution fresh for each assay, about 10 minutes before adding it to the plate.

Follow the steps below to prepare the detection antibody working solution.

Step	Action
1	Remove the PrismaA ELISA kit detection antibody (100×) vial from the refrigerator and allow it to reach room temperature (about 10 minutes). Note: <i>Protect the detection antibody from prolonged exposure to light.</i>
2	Dilute the PrismaA ELISA kit detection antibody (100×) with the PrismaA ELISA kit detection antibody diluent (1×). - For a complete 96-well plate, dilute 120 µL of the PrismaA ELISA kit detection antibody with the PrismaA ELISA kit detection antibody diluent to 12 mL. - or - For a partial plate, see Detection antibody dilution for partial plates, on page 8.
3	Mix the detection antibody working solution by gently inverting the tube 3–5 times.

Detection antibody dilution for partial plates

Number of wells	16	24	32	40	48	56	64	72	80	88	96
Vol. of detection antibody stock (µL)	20	30	40	50	60	70	80	90	100	110	120
Vol. of antibody diluent (mL)	2	3	4	5	6	7	8	9	10	11	12

5 Sample preparation and matrix effect

Introduction

The performance of the kit was tested in various sample buffers (matrices) commonly used in the purification of biotherapeutics. Some buffer systems can greatly affect the assay, causing low recovery because of suboptimal conditions, such as a high salt concentration or a high pH.

Sample dilution

Optimal dilution factors for each test sample must be determined by the user. It is recommended to perform an initial dilution of the test sample to a minimum ratio of 1:5 with Prisma ELISA kit dissociation buffer.

After dissociation, perform a two-fold serial dilution of the sample solution with dissociation buffer.

Note: *For high pH buffers, a higher dilution might be required. Determine the required dilution by performing matrix effect tests in process-specific buffers. See the following section for details.*

Note: *It is recommended that the IgG concentration in the initial sample is equal to or below 20 mg/mL. Higher concentration of IgG can affect the dissociation of the sample. Samples should be reduced to a maximum IgG concentration of 20 mg/mL by diluting in the same process-specific buffer in which the sample is contained (e.g. PBS).*

Note: *Samples containing monoclonal antibodies with a high-affinity VH3 domain might require heat-assisted dissociation. The maximum recommended IgG concentration for heat-assisted dissociation is 10 mg/mL. For detailed instructions, see [Heat-assisted dissociation, on page 12](#).*

Tip: *Centrifuge the sample to remove insoluble protein aggregates.*

Matrix effect

Matrix effects from sample specific matrices can be measured by spiking known concentrations of Prisma into test sample buffers and calculating the percentage of expected recovery. Recovery should be 80% to 120%.

If the recovery is outside of the expected range, a different proportion of dissociation buffer should be tested with the matrix.

To prepare a spiked control sample containing IgG, first pre-incubate Prisma and IgG in an appropriate process buffer for 20 minutes to allow interaction.

6 Assay procedure

Introduction

The assay procedure takes approximately 2 hours with a detection range of 0.018 to 4.5 ng/mL.

For a reproducible assay, follow the recommendations below:

- Mix the samples and reagents before use.
- Perform the sample preparation steps in a set-up plate, such as a 600 µL deep-well microplate, prior to transferring to the ELISA plate.
- Measure all standards and samples in duplicate or triplicate.
- Perform the washing procedure with the recommended wash buffer volumes.
- Do not mix material from different lots.

Prerequisites

- The reagents and plate must be at room temperature (20°C to 24°C) before starting the procedure.
- The user is responsible to validate the performance of the assay at temperatures lower than 20°C or higher than 24°C.

Note: This assay was validated at a room temperature of approximately 22°C.

Prepare the samples

The MabSelect Prisma ligand as well as other protein A derived ligands, have a high affinity for IgG. This interaction must be dissociated for the Prisma specific antibody to bind to the residual ligand present in the sample.

Follow the steps below to dissociate and prepare the samples for the assay.

Note: Some samples containing monoclonal antibodies with a high-affinity VH3 domain may require heat-assisted dissociation. See [Heat-assisted dissociation, on page 12](#) for instructions.

Tip: Perform the steps below in a deep-well plate.

Step	Action
1	Add 5 parts of Prisma Elisa kit dissociation buffer (1×) to 1 part of sample to prepare the sample solution. Tip: For example, add 400 µL of dissociation buffer to 80 µL of sample.

Step	Action
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- | | |
|---|--|
| 2 | Prepare the assay standard A by adding 8 μL of assay standard stock solution to 392 μL of dissociation buffer. |
| 3 | Incubate the sample solution and assay standard A for 20 minutes at room temperature. |
| 4 | Perform a 2.5 fold serial dilution of the assay standards in dissociation buffer, according to the table below. The volumes shown in the table are sufficient for two replicates (100 μL per well). |

Assay standard	Vol. of dissociation buffer (μL)	Vol. and source of assay standard (μL)	Assay standard concentration (ng/mL)
A	392	8 of stock	4.500
B	240	160 of A	1.800
C	240	160 of B	0.720
D	240	160 of C	0.288
E	240	160 of D	0.115
F	240	160 of E	0.046
G	240	160 of F	0.018
H	240	0	0

- | | |
|---|---|
| 5 | Perform a two-fold serial dilution of the sample solution in dissociation buffer. |
|---|---|

Tip:

To test a sample with unknown PrismA concentration, prepare an eight-step, two-fold serial dilution for the initial test. This helps determining the sample dilution that is within the dynamic range of the assay. For subsequent repeats with the same sample, a serial dilution of three or four steps can be used.

Tip:

For example, perform a serial dilution with 240 μL of dissociation buffer to yield enough material for duplicates of 100 μL per well.

Step	Action
6	Add 0.5 volumes of Prisma ELISA kit assay buffer (1×) to each sample and standard dilutions. Mix by pipetting. Note: <i>It is not necessary to adjust the concentrations of the standard curve to reflect this additional dilution.</i> Tip: <i>For example, add 120 µL of assay buffer to 240 µL of dissociated sample.</i>

Continue the procedure by following the instructions on [Capture residual Prisma on the ELISA plate, on page 13](#).

Heat-assisted dissociation

Some samples containing monoclonal antibodies with a high-affinity VH3 domain might require heat-assisted dissociation. Follow the steps below to perform heat-assisted dissociation.

Note: *For accurate results, standard dilution A must also be heat treated when using this method.*

Step	Action
1	Dilute the sample by adding 5 parts Prisma ELISA kit dissociation buffer (1×) to 1 part of sample. Note: <i>For heat-assisted dissociation the maximum recommended IgG concentration is 10 mg/mL.</i>
2	Prepare the assay standard A by adding 8 µL of assay standard stock solution to 392 µL of dissociation buffer.
3	Heat the sample solution and standard A to 95°C for 5 minutes.
4	Centrifuge the sample at 10 000 x g for 4 minutes to remove any insoluble proteins.
5	Proceed with the sample preparation in the previous section from step 4 on page 11 .

Capture residual PrismaA on the ELISA plate

Step	Action
1	Transfer 100 µL of each sample and standard dilutions to the ELISA plate.
2	Cover the plate with the plate sealer.
3	Incubate the plate on a plate shaker at 100 rpm for 30 minutes, at room temperature.
4	Prepare the detection antibody working solution before the end of the incubation. See Prepare the detection antibody working solution, on page 8 .
	Note: <i>Prepare the detection antibody working solution fresh for each assay, just before adding it to the plate.</i>

Wash the ELISA plate

It is recommended to use an automatic plate washer for washing the ELISA plate. Wash each well with 350 µL of wash buffer (1×) and soak for 10 seconds between each wash.

Tip: *If the volume of wash buffer (1×) is not sufficient, a solution of PBS + 0.05% (v/v) Tween-20 can be used.*

Note: *Complete removal of the final wash buffer is critical for optimal assay performance.*

To wash the plate manually, follow the steps below.

Step	Action
1	Remove the plate sealer.
	Note: <i>Save the plate sealer for use in subsequent steps of the procedure.</i>
2	Discard the well contents into a sink.
	Note: <i>Do not keep the wells without liquid for more than one minute.</i>
3	Add 300 µL of wash buffer (1×) per well and incubate for 10 seconds. Discard the well contents into a sink.
4	Repeat the washing procedure two more times.

Step	Action
5	After the final wash, tap the plate upside down against absorbent paper to remove any remaining wash buffer. Note: <i>Complete removal of the final wash buffer is critical for optimal assay performance.</i> Note: <i>Do not keep the wells without liquid for more than one minute.</i>

Detect the MabSelect Prisma ligand

Make sure to prepare the detection antibody working solution before the end of the sample incubation with assay buffer. See [Prepare the detection antibody working solution, on page 8](#).

Step	Action
1	Add 100 µL of the detection antibody working solution to each well and cover the plate with the plate sealer.
2	Incubate the plate on a plate shaker at 100 rpm for 30 minutes, at room temperature.
3	Wash the plate as described in the previous section, for a total of four washes. See Wash the ELISA plate, on page 13. Note: <i>After the final wash, wipe the underside of the plate to remove any residual liquid that might be present from the washing procedure.</i>
4	Add 100 µL of Prisma ELISA kit TMB to each well and cover the plate with the plate sealer.
5	Incubate the plate in the dark for 15 minutes, at room temperature. Note: <i>Do not shake the plate during incubation.</i>
6	Add 100 µL of Prisma ELISA kit stop buffer to each well to stop the reaction.
7	Read the plate using a spectrophotometer set at 450 nm wavelength with the reference wavelength at 630–650 nm to normalize the signal. Note: <i>Read the plate within 15 minutes after addition of stop buffer. Prolonged incubation might reduce the signal.</i>

Determine the PrismA concentration

Follow the steps below to determine the PrismA concentration of the test samples.

Step	Action
1	Calculate the mean Relative O.D. 450 of the replicates for each standard solution. Note: <i>If the spectrometer used for the assay does not automatically subtract the reference wavelength, do this manually prior to proceeding with the calculations.</i>
2	Plot the standard solutions data as mean Relative O.D. 450 for each standard solution (Y) vs logarithm of the respective concentration of the standard solutions (X).
3	Fit the standard solution data with a 4-parameter logistic (4-PL) curve. Tip: <i>To improve interpolation where the concentration of residual PrismA in the test samples is approaching the LOQ of the assay, perform a weighted 4-PL fit using the function $1/y^2$.</i>
4	Determine the PrismA concentration of the test samples using interpolation from the standard curve.
5	Multiply the interpolated test sample concentrations by a factor of six to account for the dilution in dissociation buffer.
6	If applicable, multiply the concentration obtained in the previous step by the sample dilution factor to give the initial concentration of each test sample.

7 Troubleshooting

Issue	Possible solutions
Low reproducibility	• Never mix components between kit lots. Each assay is lot-specific and designed for optimal performance when used before the assigned expiration date. • Review washing step procedure. Add recommended volumes of wash buffer to all wells. • Review preparation and mixing of the samples procedures. Make sure that the samples are well mixed and not aggregated. • Make sure to have proper reagent temperatures throughout the assay. • Liquid on the underside of the plate. Carefully dry the underside of the plate with an absorbent towel.
High background	• Review washing step procedure. Add recommended volumes of wash buffer to all wells. • Review the assay incubation procedure. Follow recommended incubation times. • Review preparation and mixing of samples procedure. Make sure that samples are well mixed and not aggregated.
Poor reactivity or intra assay-precision	• Review preparation and the mixing of samples procedure. Make sure that the samples are well mixed and not aggregated. • Review matrix effect: high concentrations of detergents, salts, pH and other matrices characteristics can influence the assay. • Use the provided dissociation buffer to dilute the sample to the minimum recommendations. • A resin other than MabSelect PrismA was used to purify the sample. For accurate results, the kit standard must be matched to the resin. • The monoclonal antibody contains a VH3 domain that has high affinity for PrismA. Use heat-assisted dissociation as described in Heat-assisted dissociation, on page 12.

Issue	Possible solutions
Unexpected level of recovery	• Reduce the IgG concentration to less than 20 mg/mL in the initial dilution of the sample, prior to dissociation. • A resin other than MabSelect PrismA was used to purify the sample. For accurate results, the kit standard must be matched to the resin. • The monoclonal antibody contains a VH3 domain that has high affinity for PrismA. Use heat-assisted dissociation as described in Heat-assisted dissociation, on page 12. • Make sure that the assay standards were prepared with a 2.5 fold serial dilution as described in Prepare the samples, on page 10.
Poor fit of the standard curve	• Review washing step procedure. Add recommended volumes of wash buffer to all wells. • Review preparation and mixing of samples procedure. Make sure that the samples are well mixed and with no protein aggregates. • Review pipetting procedure. Make sure that the samples are pipetted into the bottom of each well. Take care not to touch the bottom of the well with the pipette tip.

Appendix A

Quick Guide

Prepare the samples

Note: Choose step **5a** for most sample types, or step **5b** for samples containing monoclonal antibodies with high-affinity VH3 domains.

Step	Task	Instruction
1	Bring the reagents and plate to room temperature (20°C to 24°C).	Prerequisites, on page 10
2	Mix the solutions thoroughly.	Prepare the samples, on page 10
3	Dilute 1 volume of each sample with 5 volumes of dissociation buffer.	
4	Prepare assay standard A by adding 8 µL of assay standard stock solution to 392 µL of dissociation buffer	
5a OR	Incubate the sample solution and standard A for 20 minutes at room temperature.	
5b	For samples requiring heat assisted dissociation, heat the sample solution and standard A to 95°C for 5 minutes. Centrifuge the sample solution and standard A for 4 minutes.	Heat-assisted dissociation, on page 12
6	Perform a 2.5 fold serial dilution of the assay standards in dissociation buffer.	Prepare the samples, on page 10
7	Perform a 2 fold serial dilution of the samples in dissociation buffer.	
8	Add 0.5 volumes of assay buffer to each sample and standard dilution. Mix by pipetting.	

Capture the ligand

Step	Task	Instruction
1	Add 100 µL of each sample and standard dilutions into designated wells in the ELISA plate.	<i>Capture residual PrismaA on the ELISA plate, on page 13</i>
2	Cover the plate and incubate on a plate shaker at 100 rpm for 30 minutes at room temperature.	

Wash the plate

Wash the plate using an automated plate washer and 350 µL of wash buffer per well. Alternatively, follow the instructions for the manual wash procedure below.

Step	Task	Instruction
1	Discard the well contents.	<i>Wash the ELISA plate, on page 13</i>
2	Wash the plate 3x with 300 µL of wash buffer (1x), soaking for 10 seconds between each wash.	
3	Discard the well contents. Tap the plate upside down onto absorbent paper to remove remaining wash buffer.	

Note: Do not keep the wells without liquid for more than one minute.

Detect the ligand

Step	Task	Instruction
1	Add 100 µL of detection antibody working solution to each well.	<i>Detect the MabSelect PrismaA ligand, on page 14</i>
2	Cover the plate and incubate on a plate shaker at 100 rpm for 30 minutes at room temperature.	
3	Wash the plate 4x as described above.	
4	Add 100 µL of TMB buffer to each well.	
5	Cover the plate and incubate in the dark for 15 minutes at room temperature.	
6	Add 100 µL of stop buffer to each well.	
7	Read the plate at 450 nm wavelength with reference wavelength at 650 nm.	

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