

VH3 ligand ELISA kit

Instructions for Use

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1 Introduction

Product code

29737000

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. Some components might produce an allergic reaction in susceptible individuals.

Storage

Store all kit components at 2°C to 8°C upon arrival.

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 VH3 ligand ELISA kit (29737000) 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™ VH3 resin.

The kit is optimized for processes using MabSelect VH3 resins. Compatibility with other protein A derived ligands has not been tested and cannot be guaranteed.

Assay description

Residual ligands of MabSelect VH3 in process-derived samples are captured in a microplate. The plate is pre-coated with a purified, polyclonal, antibody that recognizes the VH3 ligand.

The captured proteins are detected using an HRP-conjugated, polyclonal, antibody that recognizes the VH3 ligand. The addition of TMB results in a chromogenic change that can be measured photometrically at 450 nm with reference wavelength at 630 to 650 nm. The optical density (O.D.) in the wells is proportional to the concentration of VH3 ligand and correlates to the amount of ligand present in the original sample. The VH3 ligand concentration can be calculated based on the calibration curve generated with the provided standard.

The detection of VH3 ligand can be affected by matrix composition and by the drug substance concentration. Users are encouraged to validate the performance with their specific drug substance and matrices.

Assay performance characteristics

The complete validation package is available in the accompanying data file available from the **Related 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 2 mg/mL IgG
Sensitivity, Lower Limit of Quantification (LLQ)	10 pg/mL in 2 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
VH3 ligand ELISA kit 96 well strip-plate	1 plate
VH3 ligand ELISA kit detection antibody (100×)	140 µL/vial
VH3 ligand ELISA kit detection antibody diluent (1×)	15 mL/bottle
VH3 ligand ELISA kit wash buffer (10×)	25 mL/bottle
VH3 ligand ELISA kit TMB (1×)	15 mL/bottle
VH3 ligand ELISA kit stop buffer	15 mL/bottle
VH3 ligand ELISA kit dissociation buffer (1×)	25 mL/bottle
VH3 ligand ELISA kit plate sealer	1 sheet
VH3 ligand ELISA kit standard, 225 ng/mL	250 µL/vial

Reagents and materials required but not provided

- Microcentrifuge tubes (0.5 or 1.5 mL)
- Single-channel pipettes (2 to 20 µL, 10 to 100 µL, and 100 to 1000 µL) and tips
- Multichannel pipette (50 to 300 µL) and tips
- Spectrophotometer (microplate reader) capable of reading 96-well plates at 450 nm with a reference filter at 630 to 650 nm
- Reagent reservoirs
- Plate shaker
- Vortex mixer
- Stir plate and magnetic stir bar
- Heating block capable of reaching 100°C
- Absorbent paper
- Timer
- Polypropylene centrifuge tubes (15 mL)
- Deionized water
- 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 wash buffer (1×)

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

Step	Action
1	Add 25 mL of VH3 ligand 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>

Assay standards preparation

A standard curve must be generated for every assay. The VH3 ligand ELISA kit standard 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 a serial dilution of the assay standards, see [Prepare the samples, on page 9](#).

Prepare the detection antibody working solution

Prepare the detection antibody working solution fresh for each assay. The working solution can be prepared up to 30 minutes prior to addition to the plate.

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

Step	Action
1	Remove the VH3 ligand 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>

Step Action

- 2

Dilute the VH3 ligand ELISA kit detection antibody (100×) with the VH3 ligand ELISA kit detection antibody diluent (1×).

 - For a complete 96-well plate, dilute 120 µL of the VH3 ligand ELISA kit detection antibody with the VH3 ligand ELISA kit detection antibody diluent to 12 mL.
 - or
 - For a partial plate, use the table below to prepare the detection antibody working solution.

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

- 3

Mix the detection antibody working solution by gently inverting the tube 3 to 5 times.

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 low 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 1:4 with VH3 ligand ELISA kit dissociation buffer.

After dissociation, follow the instructions in [Prepare the samples, on page 9](#).

Note: *For buffers that are very acidic ($\text{pH} < 3$) or very basic ($\text{pH} > 10$), 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: *The assay has been validated in samples with up to 30 mg/mL of drug substance. Higher concentrations can affect the recovery due to incomplete dissociation.*

Matrix effect

Matrix effects from sample specific matrices can be quantified by adding known concentrations of VH3 ligand into test sample buffers and calculating the recovery expressed as percentage of nominal VH3 concentration. Recovery should be 80% to 120%.

To prepare a spiked control sample containing drug substance, first pre-incubate the VH3 ligand and drug substance in an appropriate process buffer for 20 minutes to allow interaction.

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

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 μ L 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.

Note: *The dissociation buffer may contain precipitates that dissolve once the solution reaches room temperature.*

- The reagents must be homogenized by inverting them at least five times 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 VH3 ligand has a high affinity for molecules containing the variable heavy chain of the VH3 sequence family. This interaction must be dissociated for the assay antibodies to bind to the residual ligand present in the sample.

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

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

Step	Action
1	Add 4 parts of VH3 ligand ELISA kit dissociation buffer (1×) to 1 part of sample to prepare the sample solution. Tip: <i>Add 400 μL of dissociation buffer to 100 μL of sample to have enough material for duplicate wells after the serial dilution.</i>

Step	Action
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Note:

A 1:1 ratio of dissociation buffer to sample can also work but must be validated by the user. Factors that influence the compatibility include the concentration of drug substance and the composition of the process buffer.

- | | |
|---|---|
| 2 | Prepare the assay standard A by adding 8 µL of VH3 ligand ELISA kit standard (225 ng/mL) to 392 µL of dissociation buffer. |
| 3 | Incubate the sample solutions and assay standard A at 95°C for 10 minutes. |
| 4 | Allow the samples and standard to reach room temperature and centrifuge briefly to remove any eventual condensate. |
| 5 | 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

- | | |
|---|---|
| 6 | Perform a two-fold serial dilution of the sample solution in dissociation buffer. |
|---|---|

Tip:

If a precipitate is observed during sample preparation, centrifuge the sample at 10 000 rcf for 5 minutes and use only the supernatant to avoid interference.

Tip:

To test a sample with unknown VH3 ligand 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.

Step	Action
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Tip:

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

Capture residual VH3 ligand on the ELISA plate

Step	Action
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|---|--|
| 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 450 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 6 . |

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 $\times$) and soak for 10 seconds between each wash. Repeat for a total of three washes.

Tip: If the volume of wash buffer (1 $\times$) 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).

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
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|---|---|
| 1 | Remove the plate sealer.

Note: Save the plate sealer for use in subsequent steps of the procedure. |
| 2 | Discard the well contents into a sink.

Note: Do not keep the wells without liquid for more than one minute. |
| 3 | Add 300 μL of wash buffer (1 $\times$) per well and incubate for 10 seconds. Discard the well contents into a sink. |

Step	Action
4	Repeat the washing procedure two more times, for a total of three washes.
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 VH3 ligand

Make sure to prepare the detection antibody working solution before the end of the sample incubation in the ELISA plate. See [Prepare the detection antibody working solution, on page 6](#).

Step	Action
1	Add 100 μ L 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 450 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 11 .
	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 μ L of VH3 ligand ELISA kit TMB to each well and cover the plate.
5	Incubate the plate in the dark for 10 minutes at room temperature.
	Note: <i>Do not shake the plate during incubation.</i>
6	Add 100 μ L of VH3 ligand 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 VH3 ligand concentration

Follow the steps below to determine the VH3 ligand 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 (A650), do this manually.</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 VH3 ligand 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 VH3 ligand concentration of the test samples using interpolation from the standard curve.
5	Multiply the interpolated test sample concentrations by a factor corresponding to the dilution factor of the sample in dissociation buffer. Note: <i>If the recommended ratio of sample to dissociation buffer is used, multiply the concentration by five.</i>
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 the washing step procedure. Add the recommended volumes of wash buffer to all wells. • Review the procedures for the preparation and mixing of the samples. 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 the washing step procedure. Add the recommended volumes of wash buffer to all wells. • Review the assay incubation procedure. Follow the recommended incubation times. • Review the procedures for the preparation and mixing of the samples. Make sure that the samples are well mixed and not aggregated.
Poor reactivity or intra assay-precision	• Make sure that the reagents have reached room temperature and are homogenous before use. • Review the procedures for the preparation and mixing of the samples. Make sure that the samples are well mixed and not aggregated. • Review the 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 VH3 was used to purify the sample. For accurate results, the kit standard must be matched to the resin. • Make sure the samples have been incubated at 95°C for 10 minutes. • Make sure any precipitate developed during heat treatment is not transferred to the assay wells. Centrifuge the sample at 10 000 rcf for 5 minutes, and transfer only the supernatant.

Issue	Possible solutions
Unexpected level of recovery	• Reduce the drug substance concentration to less than 30 mg/mL in the initial dilution of the sample, prior to dissociation. • A resin other than MabSelect VH3 was used to purify the sample. For accurate results, the kit standard must be matched to the resin. • Make sure that the assay standards were prepared with a 2.5 fold serial dilution as described in Prepare the samples, on page 9.
Poor fit of the standard curve	• Review the washing step procedure. Add the recommended volumes of wash buffer to all wells. • Review the procedures for the preparation and mixing of the samples. Make sure that the samples are well mixed and not aggregated. • Review the 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

Step	Task	Instruction
1	Bring the reagents to room temperature.	Prerequisites, on page 9
2	Prepare the wash buffer.	Prepare the wash buffer (1×), on page 6
3	Prepare the samples by adding 1 part of sample to 4 parts of dissociation buffer.	Prepare the samples, on page 9
4	Prepare the standard A by mixing 8 µL of the kit standard (225 ng/mL) to 392 µL of dissociation buffer.	Prepare the samples, on page 9
5	Incubate the samples and standard A at 95°C for 10 minutes.	Prepare the samples, on page 9
6	Prepare the remaining assay standards by doing a 2.5-fold serial dilution in dissociation buffer.	Prepare the samples, on page 9
7	Prepare the samples by doing a 2-fold serial dilution in dissociation buffer.	Prepare the samples, on page 9
8	Transfer 100 µL of each sample and standard dilutions into duplicate wells of the ELISA plate.	Capture residual VH3 ligand on the ELISA plate, on page 11
9	Cover the plate and incubate on a plate shaker at 450 rpm for 30 minutes.	Capture residual VH3 ligand on the ELISA plate, on page 11
10	Dilute the detection antibody (100×) in antibody diluent.	Prepare the detection antibody working solution, on page 6
11	Wash the wells three times with 350 µL of wash buffer (1×).	Wash the ELISA plate, on page 11
12	Add 100 µL of detection antibody to each well and incubate on a plate shaker at 450 rpm for 30 minutes.	Detect the VH3 ligand, on page 12
13	Wash the wells four times with 350 µL of wash buffer (1×).	Wash the ELISA plate, on page 11
14	Add 100 µL of TMB to each well and incubate in the dark for 10 minutes.	Detect the VH3 ligand, on page 12
15	Add 100 µL of stop buffer to each well and measure the absorbance at A450–A650.	Detect the VH3 ligand, on page 12

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