VH3 ligand ELISA kit

BIOPHARMACEUTICAL IMPURITY TESTING

The VH3 ligand ELISA kit is an enzyme-linked immunosorbent assay designed to measure residual ligand leakage when using MabSelect™ VH3 protein A chromatography resin. The kit is used during the development and manufacture of bispecific monoclonal antibody (bsAb) and antibody-fragment biopharmaceuticals containing a VH3-family heavy chain domain.

Purification of antibody-based therapeutics is routinely performed using protein A-based affinity chromatography, which provides high capacity and consistent purification performance. When using protein A-based purification techniques, it is a regulatory requirement to measure leached protein A ligand in the generated downstream material. For the most accurate results, it is important that manufacturers use a resin-matched standard to calibrate their assay. This commercial ELISA kit is specifically designed for use with MabSelect VH3 protein A resin. The kit (Fig 1) contains all the necessary reagents required, including the VH3 ligand. We developed this kit with a focus on usability, robustness, and environmental sustainability.

Key benefits

- **Matched VH3 protein A ligand included in the kit** removes the need to source the standard separately and create a custom protocol.
- **Parts per billion (ppb) sensitivity** minimizes the risk of undetected residual VH3 ligand.
- **High drug product tolerance** simplifies sample preparation.
- **Consistently low intra- and inter-plate variability** ensures reproducible data.
- **All-in-one kit** containing all reagents needed to perform the assay.

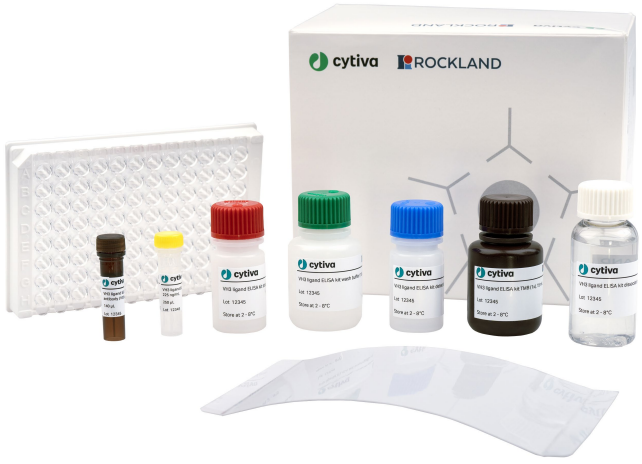

Fig 1. VH3 ligand ELISA kit.

Start-to-finish sample compatibility with bispecific antibodies

When developing a new downstream process for the purification of a therapeutic antibody product, it is good practice to demonstrate clearance of impurities, such as protein A, at each process step. This enables optimizations of the process to be made in response to changes in the detected impurities. It is therefore crucial to have a protein A assay that can consistently detect residual ligand in a multitude of different sample types. To demonstrate this, we sampled representative fractions from the purification of a therapeutic bsAb using a standard downstream purification process. MabSelect VH3 protein A resin was used for the affinity step, Capto™ S ImpAct resin for the first polishing step and cation exchange, and Capto Q resin for the second polish step and anion exchange (1). The material from the second polish step was then concentrated and buffer exchanged using a size exclusion spin filter.

To measure compatibility, we tested samples from each purification step for residual VH3 ligand in triplicate assays across several dilutions using the VH3 ligand ELISA kit. We also spiked the kit protein standard into a later downstream process step to act as a control. We interpolated the measured absorbance values for each sample to concentration (in pg/mL) using a weighted ($1/y^2$) 4-PL fit of the standard curve and converted the average to ppm. We excluded absorbance values from samples that were outside the assay specifications. The results indicated the VH3 ligand ELISA kit had good compatibility with each of the process steps, as seen by the parallel response in the calculated stock concentration (Fig 2) and accurate recovery in the spiked control sample (Table 1).

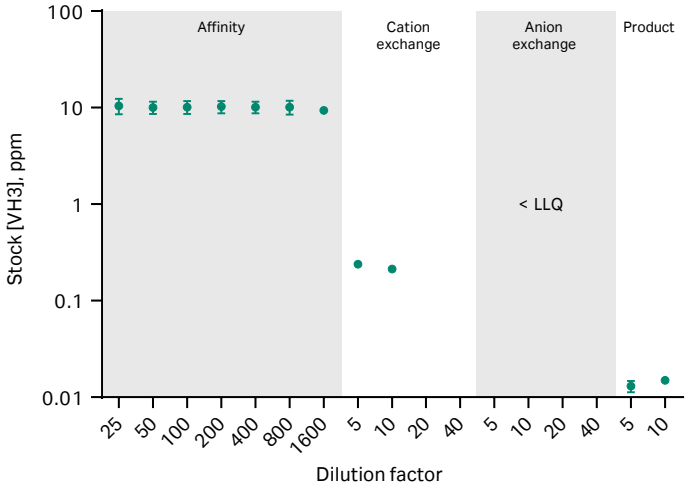

Fig 2. Parallelism of residual VH3 ligand across in-process purification steps for a bsAb. Error bars represent standard deviation from replicate experiments. Anion exchange samples were below the lower limit of quantitation (LLQ).

Table 1. Calculated concentration of residual VH3 ligand (ppm) across in-process purification steps for a bsAb

Process step	VH3 ligand in ppm (ng/mg)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
Affinity	10.206	15.23	3.29	7/7
Cation exchange	0.226	3.53	8.36	2/4
Anion exchange	< LLQ	n/a	n/a	0/4
Product	0.013	13.99	8.34	2/4
Cation exchange (spike)	111.40% recovery	0.51	N/A	N/A

Accurate results with challenging process samples

Antibody fragments represent an emerging new class of biologic therapeutics. These molecules can be particularly challenging to work with due to their small size and often enhanced affinity for protein A. As with standard mAb process development, it is good practice to demonstrate clearance of possible impurities at each process step to aid process optimization. This can be especially challenging with very high-affinity fragments such as single domain antibodies (dAb).

MAbs having two high-affinity VH3 domains can also bind very strongly to MabSelect VH3 resin. This enhanced binding can make it difficult to dissociate leached ligand from the drug substance.

To demonstrate compatibility of the VH3 ligand ELISA kit with a dAb and mAb, we sampled representative fractions from the purification of both a therapeutic VH3-family dAb and a mAb containing two high-affinity VH3 domains using a standard downstream purification process. This comprised of affinity purification using MabSelect VH3 protein A resin, Capto™ S ImpAct resin polishing, Capto™ Q resin polishing and concentration and buffer exchange using size exclusion spin filters.

To measure compatibility with these samples, purification steps were tested for residual VH3 ligand in triplicate assays across several dilutions using the VH3 ligand ELISA kit. We also spiked the kit protein standard into a later downstream process step to act as a control. We interpolated the measured absorbance values for each sample to concentration (in pg/mL) using a weighted ($1/y^2$) 4-PL fit of the standard curve and converted the average to parts per million (ppm) relative to the concentration of the drug substance. We excluded absorbance values from samples that were outside the standard curve range. The results indicated the VH3 ligand ELISA kit worked well with these challenging samples and had good compatibility with each of the process steps. This is demonstrated by the parallel response in the calculated stock concentration (Fig 3 and 4) and accurate recovery in the spiked control sample (Table 2 and 3).

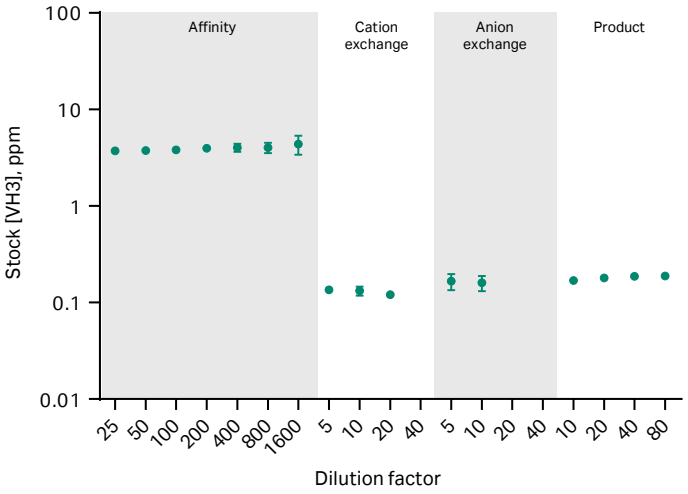

Fig 3. Parallelism of residual VH3 ligand across in-process purification steps for a dAb. Error bars represent standard deviation from replicate experiments.

Table 2. Calculated concentration of residual VH3 ligand (ppm) across in-process purification steps for a dAb

Process step	VH3 ligand in ppm (ng/mg)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
Affinity	3.961	8.76	5.69	7/7
Cation exchange	0.129	5.33	6.18	3/4
Anion exchange	0.164	18.92	0.51	2/4
Product	0.181	3.08	4.62	4/4
Cation exchange (spike)	105.42% recovery	7.99	N/A	N/A

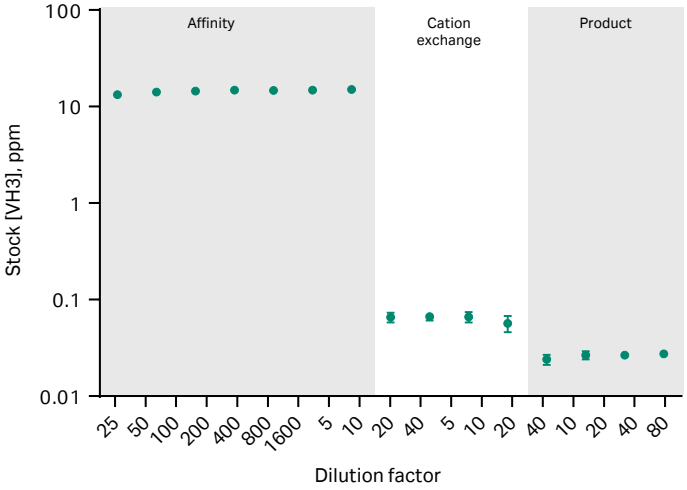

Fig 4. Parallelism of residual VH3 ligand across in-process purification steps for a mAb containing two high-affinity VH3 domains. Error bars represent standard deviation from replicate experiments.

Table 3. Calculated concentration of residual VH3 ligand (ppm) across in-process purification steps for a mAb containing two high-affinity VH3 domains

Process step	VH3 ligand in ppm (ng/mg)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
Affinity	13.842	4.45	3.99	7/7
Cation exchange	0.065	7.79	1.43	4/4
Product	0.026	5.50	3.69	4/4
Cation exchange (spike)	103.57% recovery	5.64	N/A	N/A

High IgG tolerance increases compatibility with late process samples

MabSelect VH3 protein A resin have a high affinity for antibodies and antibody fragments containing a VH3-family domain. This is also true of leached protein A ligand and as such, the interaction between leached ligand and drug product must be dissociated prior to testing so that the assay antibodies may bind to the leachate in the ELISA. This dissociation process relies on high temperature to disrupt VH3 ligand binding. Higher concentrations of drug product, typical in downstream samples, can be difficult to dissociate, especially when the concentration of protein A ligand is very low (< 1 ppm) due to the large excess of drug product. To determine the effect of IgG on dissociation, we spiked high, medium, and low concentrations of VH3 ligand into concentrations of IgG from 6 to 40 mg/mL. We then measured recovery of VH3 ligand in the VH3 ligand ELISA kit. We were able to accurately quantify the VH3 ligand within the assay specification (80 to 120% recovery) in samples containing IgG up to 30 mg/mL (Table 4). The largest ratio of VH3 ligand to IgG that passed the test criteria was 30 mg/mL recombinant IgG spiked with 150 pg/mL VH3 ligand. The recovery of ligand from this sample was 82.84%, indicating an assay sensitivity of 0.005 ppm or 5 ppb. The high tolerance to IgG means that late-process samples do not require extensive dilution to be compatible with the ELISA kit. As leached ligand content is typically very low in late-process samples, this minimal dilution increases the likelihood that the samples remain within the dynamic range of the assay and can be measured. As each drug substance may have a different affinity for the VH3 ligand, you should perform similar experiments with your in-process samples to confirm the drug product tolerance of the ELISA kit.

Table 4. IgG tolerance (recoveries outside of the acceptance criteria are shown in italicized text)

	VH3 ligand (pg/mL)					
	15 000		2500		150	
IgG (mg/mL)	ppm	Recovery (%)	ppm	Recovery (%)	ppm	Recovery (%)
40	0.38	*78.73*	0.06	88.52	0.004	*74.36*
30	0.50	102.16	0.08	89.49	0.005	82.84
20	0.75	99.21	0.13	93.88	0.008	87.68
15	1.00	102.77	0.17	97.15	0.010	91.65
10	1.50	102.37	0.25	95.18	0.015	87.34
6	2.50	99.75	0.42	94.66	0.025	82.82

High sensitivity minimizes the risk of undetected ligand

Leached protein A concentration in downstream materials is usually very low, especially at later stages of purification. It is therefore important to have a highly sensitive assay that can detect minute amounts of ligand.

The lower limit of detection (LLD) is the lowest concentration that can be distinguished from the assay background. The lower limit of quantitation (LLQ) is the lowest concentration that can be measured reliably and reproducibly. To calculate the LLD and LLQ, we spiked known concentrations of VH3 ligand protein standard into IgG, dissociated the complex and measured absorbance and recovery of nine replicate samples for each concentration across three plates. Each spiked sample contained a final concentration of 2.0 mg/mL IgG after dissociation. We calculated LLD as the concentration for which the signal was greater than three standard deviations (σ) from the mean of the zero standard [0.01022 + (3 × 0.00033) = 0.01122] (Table 5). For each replicate, we calculated LLQ as the lowest concentration for which the coefficient of variation (CV) was less than 20% and recovery was between 70% and 130%. The average result from three assays is summarized in Table 6.

Table 5. LLD calculation

Concentration of VH3 ligand (pg/mL)	60	50	40	30	20	10	0
Mean A450	0.05300	0.04556	0.03756	0.03089	0.02367	0.01700	0.01022
Standard deviation (σ)	0.00100	0.00133	0.00100	0.00133	0.00033	0.00067	0.00033
Blank + 3σ							0.01122
Mean abs. > blank + 3σ	Yes	Yes	Yes	Yes	Yes	Yes	

Table 6. LLQ calculation

Concentration of VH3 ligand (pg/mL)	60	50	40	30	20	10
Mean interpolated concentration (pg/mL)	56.65	46.21	35.92	27.26	17.77	9.16
Average recovery (%)	92.75	92.43	89.79	90.85	88.85	91.55
Average CV (%)	1.99	3.53	3.27	6.20	3.16	8.16

We then used spike assays to confirm the LLD and LLQ, testing a total of 72 replicates at 10 pg/mL in the presence of 2.0 mg/mL IgG across three plates (Fig 5). For LLD, the average absorbance from 72 replicates of the blank plus 3σ was 0.0102, and the average absorbance from 72 replicates of the 10 pg/mL standard was 0.0167. This indicates an LLD of less than 10 pg/mL. For LLQ, the CV of 72 replicates of the 10 pg/mL standard was 9.90% with a mean recovery of 97.14% This indicates an assay LLQ of around 10 pg/mL in the presence of 2.0 mg/mL IgG.

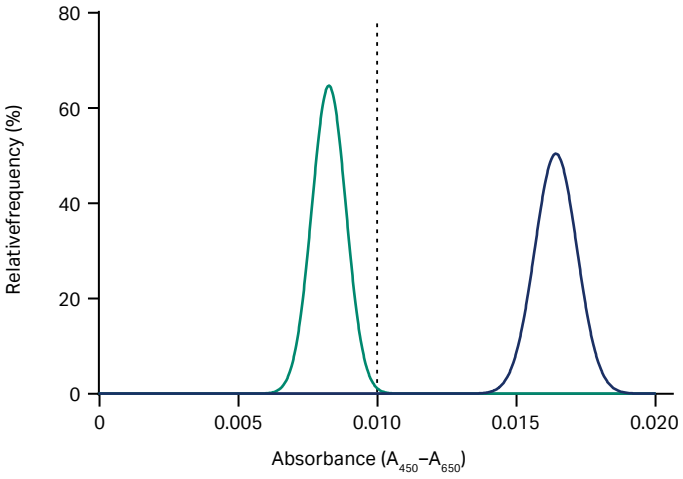

Fig 5. Distribution of absorbances from 72 replicates of the assay blank (green) and 10 pg/mL standard (blue) in 2 mg/mL IgG. The mean blank absorbance plus three standard deviations is indicated by the dotted line.

A robust and reproducible assay gives confidence in data

To have the greatest confidence in your data, it is important that your assay can give the same results over multiple experiments. Assay precision is the coefficient of variation (CV) within a single assay (intra-assay) and across several experiments (inter-assay).

To analyze intra-assay variation, we measured the CV from the interpolated concentration of 155 in-process sample and spiked sample measurements. 89% of samples had an intra-assay CV < 5%, with a mean CV of 2.10% (Fig 6). To perform inter-assay precision analyses, spiked samples containing 2500, 1000, 500 or 50 pg/mL VH3 ligand, with and without IgG were tested in the assay. We then calculated inter-assay precision from the averaged mean recovery of three replicate assays (Table 7).

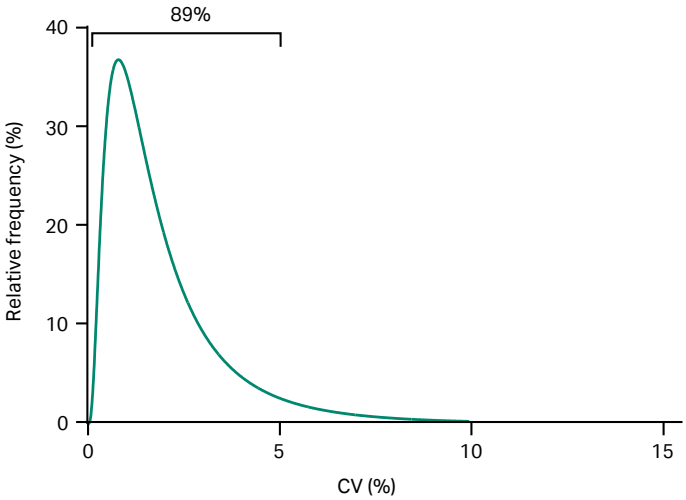

Fig 6. Intra-assay variation (CV%) from 155 replicate sample measurements.

Table 7. Inter-assay precision

VH3 Ligand (pg/mL)	IgG (mg(mL)	Average recovery (%)	CV (%)
2500	–	98.62	2.05
2500	2.0	103.24	5.15
1000	–	91.30	1.96
1000	2.0	91.82	15.04
500	–	91.38	3.55
500	2.0	101.61	5.03
50	–	94.01	14.16
50	2.0	102.41	19.27

Wide buffer compatibility for use across multiple processes

A robust assay should be compatible with all downstream buffers used in a particular process. This removes the need for complicated and time-consuming buffer exchange protocols that may compromise the integrity of the samples. To test for possible buffer effects (also called matrix effects), we performed spike recovery assays with buffers commonly used in mAb purification. We spiked IgG into five different downstream process buffers with 1 ppm VH3 ligand, then followed the standard dissociation protocol as described in the instruction for use. Each initial spiked sample contained a final concentration of 1.2 mg/mL IgG after dissociation. We serially diluted the samples two-fold over eight dilutions in dissociation buffer, then measured recovery of VH3 ligand using ELISA. This assay was performed across triplicate experiments. The pass criterion for recovery was an interpolated concentration at each dilution that was between 80% and 120% of the expected result. We then calculated parallelism of the interpolated stock concentration, which is defined as the CV across all tested dilutions. The pass criterion for parallelism was a CV < 20% of the interpolated stock concentration across all dilutions. The results showed that the VH3 ligand ELISA kit was compatible with all five buffers tested, as indicated by all dilutions of each sample (8/8) being within the acceptance criteria of the assay, and the parallelism being below the target of 20% (Table 8). For optimal performance, we recommend diluting test samples 1:4 in dissociation buffer, and then performing a serial dilution into dissociation buffer after the initial incubation, as shown

in the product instructions. We also recommend routinely measuring the recovery of a spiked sample to detect any process-specific matrix effects. If you discover any precipitates or aggregates in your test samples, centrifuge them to remove insoluble proteins that may cause complications in the assay.

Table 8. Matrix testing

Matrix	Average recovery (%)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
50 mM sodium acetate, pH 3.5	100.24	3.82	3.43	8/8
25 mM sodium phosphate, pH 7.5	93.08	4.43	4.15	8/8
100 mM glycine, pH 3.5	101.06	3.89	2.19	8/8
100 mM Tris-citrate, pH 7.5	95.93	7.59	5.88	8/8
100 mM Tris-acetate, pH 7.5	99.26	4.24	8.28	8/8

A broad dynamic range simplifies sample preparation

The VH3 ligand ELISA kit has a broad dynamic range that helps to reduce the time and guesswork needed to dilute samples into the assay range. We used the protein standard from the kit to prepare a 2.5-fold standard calibration curve from 4.5 ng/mL to 18 pg/mL (Fig 7). The standard curve was measured in triplicate wells across six assays (18 replicates total) and showed reproducible interpolation of the standard (80 to 120%) with a strong goodness of fit ($R^2 > 0.999$) (Table 8). The absorbances shown here are representative results from newly manufactured assays and are not intended to demonstrate target absorbances for a functioning assay. Absolute absorbance can be affected by multiple factors including ambient temperature, effectiveness of the washing procedure and exact incubation times, especially with chromogenic substrate (i.e., TMB). Note: the kit absorbance values are affected over time and will reduce from the maximum value as the kits age from the date of manufacture.

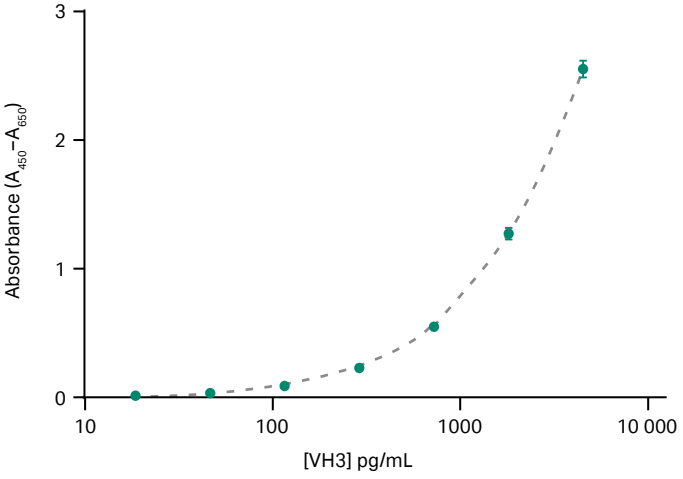

Fig 7. Standard curve (weighted 4-PL fit) of the VH3 ligand ELISA kit. Error bars represent the standard deviation from six replicate experiments.

Table 9. Mean absorbance mean recovery, and inter-plate CV from six replicates of the standard curve

VH3 ligand (pg/mL)	Mean absorbance (A_{450}-A_{650})	Mean recovery (%)	CV recovery (%)
4500	2.559	100.06	0.828
1800	1.281	100.29	1.073
780	0.558	98.88	1.576
288	0.236	101.15	1.669
115.20	0.099	99.61	0.862
46.08	0.045	100.32	1.429
18.43	0.023	99.55	2.217

Sustainability designed in

At Cytiva, we have a mission to reduce our environmental impact, and help our customers to do the same. With this in mind, we have taken extra care during development to address sustainability in our VH3 ligand ELISA kit. We have used recyclable paper packaging, and the plastics included can be recycled where facilities exist.* We have optimized the volumes and concentrations of buffers provided to decrease the overall shipping weight, reducing our shipping carbon footprint. To reduce paper usage, the user documentation (instructions for use) is in a digital format, which can be easily accessed by scanning the QR code on the product label. Finally, we have used a biodegradable and environmentally friendly surfactant which complies with the tenth principle of green chemistry (design for degradation).

* We recommend that the 8-well strips of the ELISA plate not be recycled and be treated as hazardous lab waste, as they may contain trace amounts of biological or hazardous material, even after washing.

Reagent stability

We used an accelerated stability study to determine shelf life. We stored components at 25°C or 37°C and tested performance at regular intervals over 56 days (37°C) or 100 days (25°C) using the criteria shown in Table 11. All components passed the accelerated stress testing at day 56/100. This indicates that the predicted shelf life of the kit is 12 months from the date of manufacture when stored between 2°C and 8°C

Table 10. Test criteria and results of the accelerated stability study

Parameter	Pass criteria	Result
Absorbance of the 1.8 ng/mL standard	> 0.7	Pass
Absorbance of the blank standard	< 0.04	Pass
Intra-assay CV	< 20%	Pass
LLD (pg/mL)	< 18	Pass
Recovery of each standard	80% to 120%	Pass

Specifications

Specification	Parameter
LLD	10 pg/mL in 2 mg/mL IgG
LLQ	10 pg/mL in 2 mg/mL IgG
Intra-assay CV	< 20%
Inter-assay CV	< 20%
Range	18 pg/mL to 4.5 ng/mL
Recovery of each standard	80% to 120%

Kit contents

Description	Quantity
VH3 ligand ELISA kit 96 well strip-plate	1 plate
VH3 ligand ELISA kit detection antibody (100x)	140 µL/vial
VH3 ligand ELISA kit detection antibody diluent (1x)	15 mL/bottle
VH3 ligand ELISA kit wash buffer (1x)	25 mL/bottle
VH3 ligand ELISA kit TMB (1x)	15 mL/bottle
VH3 ligand ELISA kit stop buffer	15 mL/bottle
VH3 ligand ELISA kit dissociation buffer (1x)	25 mL/bottle
VH3 ligand ELISA kit plate sealer	1 sheet
VH3 ligand ELISA kit standard, 225 ng/mL	250 µL/vial

Ordering information

Description	Product code
VH3 ligand ELISA kit	29737000

Related products

Description	Size	Product code
HiTrap™ MabSelect VH3	1 × 1 mL column	17549351
HiTrap MabSelect VH3	5 × 1 mL column	17549352
HiTrap MabSelect VH3	1 × 5 mL column	17549353
HiTrap MabSelect VH3	5 × 5 mL column	17549354
HiScreen™ MabSelect VH3	1 × 4.7 mL column	17549315
MabSelect VH3 resin	25 mL	17549301
MabSelect VH3 resin	200 mL	17549302
MabSelect VH3 resin	1 L	17549303
MabSelect VH3 resin	5 L	17549304
MabSelect VH3 resin	10 L	17549305
PreDictor™ RoboColumn MabSelect VH3, 200 µL	1 × 8 µL columns	17549333
PreDictor RoboColumn MabSelect VH3, 600 µL	1 × 8 µL columns	17549334
PreDictor MabSelect VH3, 2 µL	1 × 4 plates	17549330
PreDictor MabSelect VH3, 20 µL	1 × 4 plates	17549331
PreDictor MabSelect VH3, 50 µL	1 × 4 plates	17549332
MabSelect VH3 validation column	1 × 15.7 mL column (10/200)	17549370

Please contact us here to request information about VH3 ligand ELISA kits.

References

1. Application note: Three-step monoclonal antibody purification processes using modern chromatography media. Cytiva, 29132569 AA (2015).
2. Data file: MabSelect VH3 resin. Cytiva, CY36703 (2023).

Related documents

- Instructions for use: VH3 ligand ELISA kit
- Regulatory support file: MabSelect VH3 resin

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