PrismA ELISA™ kit

PROTEIN A LIGAND LEAKAGE DETECTION

The PrismA ELISA™ kit is an enzyme-linked immunosorbent assay (ELISA) designed to measure residual ligand leakage when using MabSelect PrismA™ and PrismA X chromatography resins and Fibro PrismA chromatography adsorbers. The kit is used during the development and manufacture of monoclonal antibody (mAb) biopharmaceuticals.

Purification of mAbs 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 kit is the only commercial ELISA kit specifically designed for use with MabSelect PrismA and PrismA X resins and Fibro PrismA adsorbers (Fig 1). The kit contains all the necessary reagents required including the PrismA ligand optimized for use in ELISA, and polyclonal antibodies raised specifically against the PrismA ligand. We developed this kit with a focus on usability, robustness, and environmental sustainability.

Key benefits

- **Matched PrismA protein A ligand included in the kit** removes the need to source the standard separately and create a custom protocol.
- **Parts per billion sensitivity** minimizes the risk of undetected residual PrismA ligand.
- **High IgG 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. Contents of PrismA ELISA kit.

High IgG tolerance

MabSelect PrismA resins and Fibro PrismA adsorbers have a high affinity for IgG, which is also true of leached protein A ligand. As such, the interaction between leached ligand and IgG must be dissociated prior to testing so that PrismA ligand-specific antibodies may bind to the leachate in the ELISA. This dissociation process relies on low pH buffers to interrupt PrismA ligand-binding to IgG in a similar mechanism to elution of IgG from protein A resin. Higher concentrations of IgG, 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 IgG.

To determine the effect of IgG on dissociation, we spiked high, medium, and low concentrations of PrismA ligand into decreasing concentrations of IgG. We then measured recovery of PrismA ligand in the PrismA ELISA kit using the standard dissociation method. We were able to accurately quantify the PrismA ligand in all samples within the assay specification (80% to 120% recovery) which demonstrates the assay is capable of detecting PrismA ligand in samples of IgG at concentrations up to 20 mg/mL (Table 1). The largest ratio of PrismA ligand to IgG tested was 20 mg/mL of recombinant IgG spiked with 180 pg/mL PrismA ligand. The recovery of ligand from this sample was 90.3%, indicating an assay sensitivity of 0.009 ppm or 9 ppb.

Table 1. IgG tolerance testing demonstrates the assay is compatible with up to 20 mg/mL IgG in the initial sample; the given concentrations of IgG and PrismA ligand in the table are pre-dissociation

	PrismA ligand (pg/mL)					
	18 000		3000		180	
IgG (mg/mL)	ppm	Recovery (%)	ppm	Recovery (%)	ppm	Recovery (%)
20	0.90	95.40	0.15	92.68	0.009	90.29
15	1.20	97.03	0.20	94.10	0.012	88.47
10	1.80	103.17	0.30	98.50	0.018	88.79
8	2.25	103.16	0.38	103.05	0.023	91.89
4	4.50	107.26	0.75	105.06	0.045	91.02
2	9.00	111.37	1.50	106.66	0.09	86.50
1	18.00	110.21	3.00	104.21	0.18	88.87
0	–	102.94	–	96.66	–	103.57

High sensitivity

Leached protein A concentration in downstream materials is usually very low, especially at the final stages of purification. It is therefore important to have a highly sensitive assay that can detect low 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 PrismA protein standard into IgG, dissociated the complex following the standard dissociation method, and measured absorbance using the ELISA kit. Each spiked sample contained a final concentration of 1.5 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 (0.01207 + [3 × 0.00036] = 0.01314) (Table 2). 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% (Table 3). We determined LLD and LLQ from nine replicates across three plates.

Table 2. LLD calculation

Concentration PrismA ligand (pg/mL)	60	50	40	30	20	10	0
Mean A_{450}	0.06373	0.05402	0.04632	0.03701	0.02903	0.02096	0.01207
Standard deviation (σ)	0.00084	0.00082	0.00073	0.00052	0.00061	0.00052	0.00036
Blank + 3σ							0.01314
Mean A_{450} > blank + 3σ	Yes	Yes	Yes	Yes	Yes	Yes	

Table 3. LLQ calculation

Concentration PrismA ligand (pg/mL)	60	50	40	30	20	10
Mean interpolated concentration (pg/mL)	65.35	53.56	44.02	32.85	22.67	12.73
Recovery (%)	106.55	104.36	106.55	104.94	106.51	113.80
CV recovery (%)	1.46	1.81	1.90	1.94	3.37	5.06

We then used spike assays to confirm the LLD and LLQ, testing a total of 80 replicates at 10 pg/mL in the presence of 1.5 mg/mL IgG across five plates (Fig 2).

For LLD, the average absorbance from 80 replicates of the blank plus 3σ was 0.0173, and the average absorbance from 80 replicates of the 10 pg/mL standard was 0.0228. This indicates an LLD less than 10 pg/mL.

For LLQ, the CV of 80 replicates of the 10 pg/mL standard was 12.45% with a mean recovery of 81.19%. This indicates an assay LLQ of around 10 pg/mL in the presence of 1.5 mg/mL IgG.

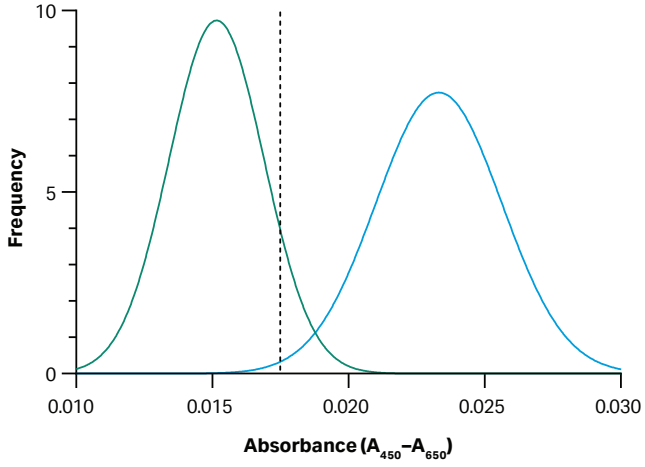

Fig 2. Distribution of absorbances from 80 replicates of the assay blank (green) and 10 pg/mL standard in 1.5 mg/mL IgG (blue). The mean blank absorbance plus three standard deviations is shown by the dashed, black line.

Start-to-finish sample compatibility

When developing a new downstream process for the purification of a mAb, 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 mAb using a standard downstream purification process using MabSelect PrismA or MabSelect PrismA X resins 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 Vivaspin sample concentrator.

To measure compatibility, we tested samples from each purification step for residual PrismA ligand in triplicate assays across several dilutions using the PrismA ELISA kit. We also spiked the kit protein standard into later downstream process steps and measured the cell culture harvest material to act as controls. We interpolated the measured absorbance values for each sample to concentration (in pg/mL) using a 4-PL fit of the standard curve and converted the average to ppm. We excluded absorbance values from samples that were outside the standard curve range.

The results indicated the PrismA ELISA kit had good compatibility with each of the process steps, as seen by the parallel response in the calculated stock concentration (Fig 3 and 4) and accurate recovery in the spiked control samples (Table 4 and 5).

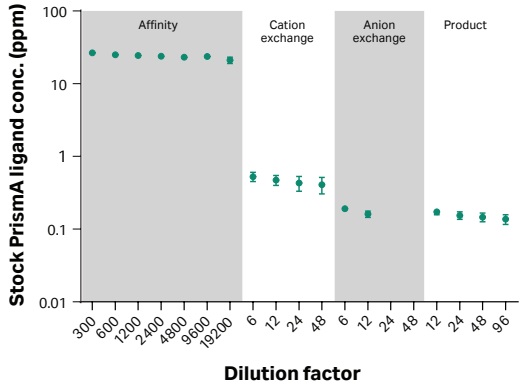

Fig 3. Parallelism of residual PrismA ligand across in-process purification steps for a mAb. Error bars represent standard deviation from replicate experiments.

Table 4. Calculated concentration of residual PrismA ligand (ppm) across in-process purification steps for a mAb

Process step	PrismA ligand in ppm (ng/mg)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
Harvest	< LOQ	–	–	–
Affinity	24.01	3.66	7.11	7/7
Cation exchange	0.46	19.21	11.43	4/4
Anion exchange	0.17	5.56	9.83	2/4
Product	0.15	12.13	9.98	4/4
Cation exchange (spike)	103.07% recovery	3.44	1.07	4/4

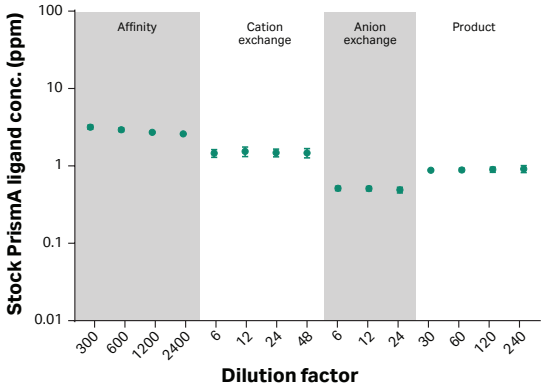

Fig 4. Parallelism of residual PrismA X ligand across in-process purification steps for a mAb. Error bars represent standard deviation from replicate experiments.

Table 5. Calculated concentration of residual PrismA X ligand (ppm) across in-process purification steps for a mAb

Process step	PrismA ligand in ppm (ng/mg)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
Affinity	2.911	8.18	6.82	4/5
Cation exchange	1.490	12.65	2.87	4/4
Anion exchange	0.505	6.24	4.10	3/3
Product	0.897	6.23	3.28	4/4

Enhanced dissociation for IgG containing high-affinity VH3 domains

For the majority of mAbs, the PrismA ligand interacts solely through binding to the Fc region. Some IgG therapeutics that utilize a VH3-family domain demonstrate an additional high-affinity binding to PrismA ligand depending upon the amino acid sequence (2). These high-affinity VH3 domain interactions with PrismA ligand are stable at the pH used to disrupt binding to the Fc region. While not affecting low pH elution from MabSelect PrismA resins in downstream purification, the VH3-mediated association with PrismA can make the standard dissociation method used in the PrismA ELISA kit less effective. To ensure a robust detection assay for this type of mAb, we developed an enhanced, heat-assisted dissociation method.

To demonstrate heat-assisted dissociation in real-world samples, we purified a therapeutic mAb containing a high-affinity VH3 domain using the standard downstream purification described above. To measure PrismA ligand in these samples, we used the heat-assisted dissociation method, then serially diluted the dissociated samples to measure PrismA ligand over multiple dilutions. We also spiked known concentrations of PrismA ligand into the later process steps and measured recovery as a control.

The results indicated the heat-assisted dissociation method is compatible with these samples, as seen by the parallel response in the calculated stock concentration (Fig 5) and accurate recovery in the spiked control samples (Table 6).

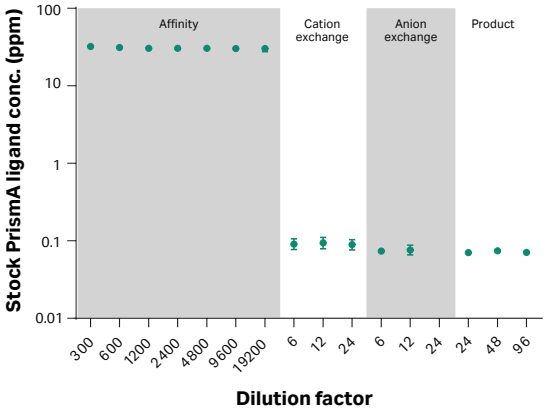

Fig 5. Parallelism of residual PrismA ligand (ppm) across in-process purification steps for a mAb containing a high-affinity VH3 domain. Error bars represent standard deviation from replicate experiments.

Table 6. Calculated concentration of residual PrismA ligand (ppm) across in-process purification steps for a mAb containing a high-affinity VH3 domain

Process step	PrismA ligand in ppm (ng/mg)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
Harvest	< LOQ	–	–	–
Affinity	30.199	9.78	4.04	7/7
Cation exchange	0.077	16.70	2.22	3/4
Anion exchange	0.066	17.77	2.35	2/4
Product	0.067	17.74	3.07	3/4
Cation exchange (spike)	114.8% recovery	12.13	3.43	4/4

A robust and reproducible assay

To have the greatest confidence in your data, it is important that your assay can reproducibly give the same results over multiple experiments. Assay precision is the 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 127 in-process sample measurements. 96% of samples had an intra-assay CV < 10%, with a mean CV of 2.88% (Fig 6). To perform inter-assay precision analysis, spiked samples containing 2500, 1000, 500, or 50 pg/mL of PrismA 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).

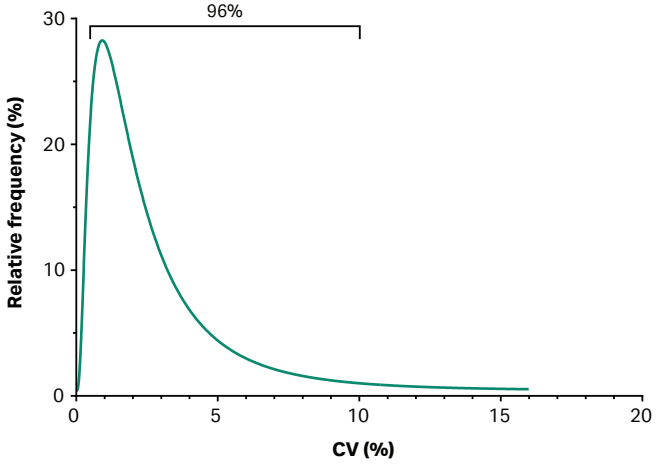

Fig 6. Intra-assay variation from 127 in-process sample measurements.

Table 7. Inter-assay precision

PrismA ligand (pg/mL)	IgG (mg/mL)	Average recovery (%)	CV (%)
2500	–	98.67	10.75
2500	1.0	103.00	7.00
1000	–	98.03	10.67
1000	1.0	102.58	6.84
500	–	108.08	17.41
500	1.0	106.09	12.94
50	–	106.41	17.42
50	1.0	114.43	15.89

Wide buffer compatibility

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 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 PrismA ligand then followed the standard dissociation protocol. Each initial spiked sample contained a final concentration of 1 mg/mL IgG after dissociation. We serially diluted the samples two-fold over eight dilutions in dissociation buffer, then measured recovery of PrismA 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 criteria for parallelism was a CV < 20% of the interpolated stock concentration across all dilutions.

The results showed that the PrismA 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 1:5 in dissociation buffer, and then performing a series dilution into dissociation buffer after the initial incubation, as shown in the product instructions.

We also recommend routinely measuring the recovery of a spike sample to detect any process-specific matrix effects. If you discover any precipitates or aggregates in your test samples (particularly if using heat-assisted dissociation) centrifuge them to remove insoluble proteins that may cause complications in the assay.

Table 8. Matrix recovery rates and good parallelism demonstrates broad buffer compatibility

Matrix	Average recovery (%)	CV of replicate experiments (%)	Average parallelism (%)	Dilutions in range
50 mM Na acetate, pH 3.5	105.06	6.31	2.80	8/8
25 mM phosphate, pH 7.5	99.47	2.57	3.17	8/8
100 mM glycine, pH 3.5	101.49	7.22	2.88	8/8
100 mM Tris-citrate, pH 7.5	101.27	6.01	2.78	8/8
100 mM Tris-acetate, pH 7.5	96.91	2.68	3.30	8/8

Broad dynamic range

The PrismA 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 duplicate wells across three assays (six replicates total) and showed reproducible interpolation of the standard, with a strong goodness of fit (R^2 > 0.999) (Table 9).

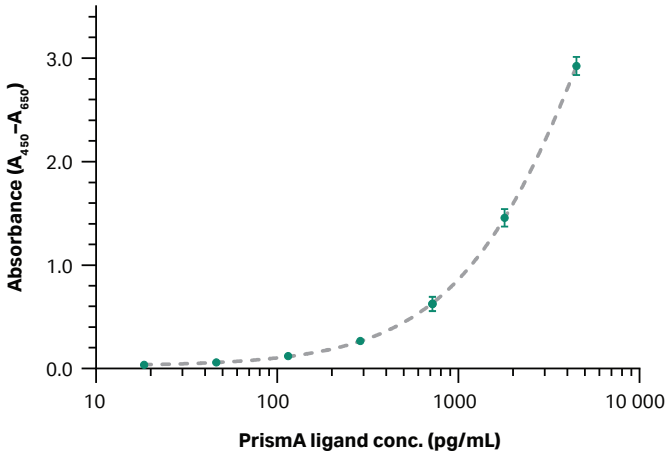

Fig7. Standard curve (4-PL fit) of the PrismA ELISA kit. Error bars represent standard deviation from replicate experiments.

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

PrismA ligand (pg/mL)	Mean absorbance (A_{450}–A_{650})	Mean recovery (%)	CV recovery (%)
4500	2.879	99.98	0.014
1800	1.444	100.18	0.175
720	0.618	99.04	1.117
288	0.261	101.57	2.612
115.2	0.109	103.52	1.235
46.08	0.049	101.77	1.965
18.43	0.026	92.58	10.161
R^2	–	0.999	–

Reagent stability

We used an accelerated stability study to determine shelf life. We stored components at 25°C and tested performance at regular intervals over 100 d using the criteria shown in Table 10. All components passed the accelerated stress testing at Day 100. This indicates that the predicted shelf life of the kit is 12 mo 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 (Day 100 at 25°C)
Absorbance of the 1.8 ng/mL standard	> 0.7	Pass
Absorbance of the blank standard	< 0.04	Pass
Intra-assay CV	< 20%	Pass
Absorbance of the 18 pg/mL standard	> Blank absorbance + 3σ	Pass
Recovery of each standard	80% to 120%	Pass

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 PrismA 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, user documentation (instructions for use) is provided in a digital format and can be easily accessed by scanning the QR code on the product label. Finally, we have switched the surfactant to a biodegradable, environmentally friendly substitute which complies with the tenth principle of green chemistry (design for degradation).

Specifications

Specification	Parameter
LLD	10 pg/mL in 1 mg/mL IgG
LLQ	10 pg/mL in 1 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

Component	Volume
PrismA ELISA kit assay buffer (1×)	10 mL
PrismA ELISA kit detection antibody diluent (1×)	15 mL
PrismA ELISA kit wash buffer (10×)	25 mL
PrismA ELISA kit TMB (1×)	15 mL
PrismA ELISA kit dissociation buffer (1×)	30 mL
PrismA ELISA kit stop buffer (1×)	15 mL
PrismA ELISA kit standard	1 vial
PrismA ELISA kit detection antibody	140 µL
PrismA ELISA kit 96 well strip-plate	1 plate
PrismA ELISA kit plate seal	1 seal

Ordering information

Item	Product code
PrismA ELISA kit	29707299

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

References

1. Application note: Three-step monoclonal antibody purification processes using modern chromatography media. Cytiva; 29132569 AA, May 2015
2. Data file: MabSelect PrismA. Cytiva; CY553-17Sep20-DF.

Related documents

MabSelect PrismA™ protein A chromatography resin
cytivalifesciences.com/shop/chromatography/resins/affinity-antibody/mabselect-prisma-protein-achromatography-resin-p-09659

MabSelect PrismA™ X chromatography resin
cytivalifesciences.com/shop/chromatography/resins/affinity-antibody/mabselect-prisma-x-protein-a-resin-p-56818

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