Version：03
Update (MM/DD/YYYY): 02/27/2025

# Product Manual

# Protein A ELISA Kit, 2G

## Cat. No. L01043

**For Research Use Only. Not for Use in Diagnostic Procedures.**

The operator should read the technical manual carefully before using this product.

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# Contents





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## I. DESCRIPTION

Protein A affinity chromatography is widely used for antibody purification. The leakage of Protein A ligands has become a significant problem in the pharmaceutical industry, even when Protein A ligands are covalently bound to chromatography support. It also probably causes false results in immunological assays if an antibody is contaminated with Protein A. Therefore, the assessment of residual Protein A is a critical quality control step in the production process of antibodies.

Leached Protein A tends to interact with residual immunoglobulins in the sample through its Fc binding domain. This interaction hinders the formation of complexes between the anti-Protein A antibodies and Protein A, thereby interfering with the accurate quantification of Protein A in immunoassays and leading to a significant underestimation of Protein A impurities. It is noteworthy that the extent of this interference varies considerably among different antibodies, with a particularly common occurrence in many human IgGs.

**GenScript Protein A ELISA Kit, 2G (Cat. No. L01043)** is designed to accurately quantify most forms of Protein A available on the market, including native Protein A, recombinant Protein A variants and Alkali-tolerant recombinant protein A variants. This kit provides a unique 'Denaturing Solution' that effectively disrupts the binding between Protein A and IgG, thereby minimizing its interference with quantification. In comparison to traditional boiling methods for Protein A dissociation, this kit offers a more accurate, simple-to-use, precise and highly sensitive method for quantifying Protein A, with a detection limit as low as 62.5 pg/mL, in mouse, rat, rabbit, or human antibody samples.

This kit can be used to measure trace amounts of Protein A in therapeutic antibody products in the pharmaceutical industry. Antibody suppliers can use the kit to evaluate Protein A containment level of the products. It also makes it easier for the manufacturers of Protein A affinity chromatography media in the process of monitoring the leaching characteristics of the media under specific conditions.

## II. ASSAY PRINCIPLE

This kit is based on the sandwich ELISA method. Standards and samples containing Protein A are first diluted in the Sample Dilution Buffer provided with the kit and the Denaturing Solution is then added and mixed to dissociate the Protein A and the antibody in the samples. Subsequently, standards or dissociated samples are added to the capture plate coated with the anti-Protein A capture antibody, allowing the Protein A ligand in the standards or samples can be captured on the plate. Then anti-Protein A detection antibody conjugated with biotin is added to interact with the Protein A ligand bound on the plate. Streptavidin-Horseradish Peroxidase conjugate (Streptavidin-HRP) is added to interact with the biotin conjugated anti-Protein A antibody. After washing steps, 3,3',5,5'-Tetramethylbenzidine solution (TMB Solution) is added to the plate and color development is stopped by the addition of stop solution. Application of the Stop Solution results in the color changing from blue to yellow. The color intensity can be read at 450 nm and 650nm by a microplate reader. The Protein A standard curve can be used to accurately quantify the amount of Protein A in the samples.



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## III. KIT CONTENTS

The kit provides the following reagents and solutions for the quantitative measurement of Protein A ligand in biological matrices.

**Table 1. Components of the kit**

| Component | Quantity/Size | Part No. |
|---|---|---|
| Capture Plate | 1 plate | Y1-80 |
| MabSelect PrismA Stock | 1 vial (0.1 mL) | Y1-10 |
| Biotin conjugated anti-Protein A Antibody | 1 bottle (12 mL) | Y1-20 |
| Streptavidin HRP | 1 bottle (12 mL) | Y1-30 |
| Sample Dilution Buffer | 1 bottle (60 mL) | Y1-60 |
| Denaturing Solution | 1 bottle (10 mL) | Y1-91 |
| Detection Buffer | 1 bottle (12 mL) | Y1-90 |
| 20×Wash Solution | 1 bottle (60 mL) | B-A1-71 |
| TMB Solution | 1 bottle (12 mL) | B-A1-40 |
| Stop Solution | 1 bottle (6 mL) | B-A1-50 |
| Plate Sealer | 2 pieces | / |

- Capture Plate: 96 well microplates (8 wells x 12 strips); 12 strips are configured in the plate; the plate is sealed in a foil pouch with a desiccant.
- MabSelect PrismA Stock contains 1 μg/mL of MabSelect PrismA ligand.
- The table above shows all components of the basic kit. For the detection of other Protein A ligands, the assay can be extended by adding alternate standards (see **Table 5. Typical assay working ranges for different sources of Protein A ligand**).
- GenScript offers the 20× Wash Solution (GenScript, B00063) if customer requires an additional wash solution.

## IV. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

## V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Protein A standards for standard curves and quality control (excluding MabSelect PrismA).
- 96-well Low Binding Microplates & Sealers(e.g. Genscript, D00042).
- Microplate reader capable of measurement at 450 nm and OD650.
- Data analysis and graphing software.
- Automated microplate washer
- Deionized or distilled water
- Graduated cylinder



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- Plastic container
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL, and 1000 µL precision pipettes and pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator
- Centrifuge
- 25°C incubator
- Rotary shaker
- Vortex Mixer

## VI. PRECAUTIONS

1. Avoid contact with skin, eyes, or clothing with Stop Solution or TMB Substrate. Keep the container tightly closed. In case of an accident, please seek medical advice immediately.
2. Do not use the kit if there is any visible damage to the packaging or kit contents.
3. Do not mix components from different batches. Do not mix with components from other manufacturers.
4. Do not use reagents beyond the stated expiry date.
5. All reagents must be equilibrated to room temperature (20-25°C) before running the assay. Only take an appropriate amount of reagents at once. Do not put unused reagents back into the vials as reagent contaminations may occur.
6. Before opening the Standard Stock, quickly span the vial to ensure that all the liquid has collected at the bottom, and prevent the liquid from splashing when opening the lid.
7. Use only distilled or deionized water and clean glassware.
8. Do not let wells dry during the test, add reagents immediately after completing washing steps.

## VII. SPECIMEN COLLECTION AND STORAGE

1. The handling and storage information provided here is intended to be used as a general guideline. When samples need to be stored for a long time, users need to evaluate the stability of the samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria that meet their needs.
2. Store samples at -20°C or lower if not tested immediately. Avoid repeated freeze-thaw cycles.



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## VIII. PROTOCOL

- **Reagent Preparation**

  All reagents must be equilibrated to room temperature before use (20-25°C). All samples and reagents should be vortexed before use. Store all reagents back in the refrigerator promptly after use.

  **1×Wash Solution:** Dilute the 20×Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 60 mL of 20×Wash Solution with 1140 mL of deionized or distilled water to make 1200 mL of 1×Wash Solution. Store the solution at 2-8°C when not in use.

  *Note: If any precipitate is found in the 20×Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.*

  **Protein A Standard Preparation:** Protein A standards should be prepared with Sample Dilution Buffer to generate eight Protein A ligand concentrations recommended in **Table 2**: 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 ng/mL, and Sample Dilution Buffer (BL).

**Table 2. Recommended standard preparation**

| Standard ID | Dilution Factor | Source (V1-10) | Source Volume (µL) | Matrix Volume (µL) | Final Volume (µL) | Final Conc. (ng/mL) |
|---|---|---|---|---|---|---|
| S-Int1 | 100 | Standard Stock | 5 | 495 | 500 | 10 |
| Std1 | 2.5 | S-Int1 | 400 | 600 | 1000 | 4 |
| Std2 | 2 | Std1 | 300 | 300 | 600 | 2 |
| Std3 | 2 | Std2 | 300 | 300 | 600 | 1 |
| Std4 | 2 | Std3 | 300 | 300 | 600 | 0.5 |
| Std5 | 2 | Std4 | 300 | 300 | 600 | 0.25 |
| Std6 | 2 | Std5 | 300 | 300 | 600 | 0.125 |
| Std7 | 2 | Std6 | 300 | 300 | 600 | 0.0625 |
| BL | 0 | / | / | 600 | 600 | 0 |

*Note: The above Protein A standard preparation process is only limited to MabSelect PrismA stock equipped in the kit. If you are using other constructs of Protein A for antibody purification, it is advisable to acquire the corresponding Protein A standard from the chromatography media supplier as a quantitative reference. This standard can be diluted in accordance with the method described above, to ensure that each concentration point of the standard curve falls within the linear range (**Table 5** lists the linear ranges of different sources of protein A standards for reference).*

- **Capture Plate Preparation**

  It is recommended that all standards, quality controls, and samples be prepared in duplicate at least. **Table 3** is an example of the setup of Protein A ligand standards and samples.

  Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.



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Leave the unused strips in the foil pouch and store at 2-8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

**Table 3. Setup of standards, quality controls and samples on Capture Plate**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | Std1 | Std1 | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 |
| **B** | Std2 | Std2 | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 |
| **C** | Std3 | Std3 | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 |
| **D** | Std4 | Std4 | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 |
| **E** | Std5 | Std5 | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 |
| **F** | Std6 | Std6 | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 |
| **G** | Std7 | Std7 | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| **H** | BL | BL | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |

Std: Standard number; S: Sample number; BL: Blank Control

- **Test Procedure**

**Sample Treatment**

1. Add 100 µL of the diluted standard solutions, quality controls and samples to the corresponding wells in the 96-well Low Binding Microplates (e.g. GenScript, D00042).
2. Add 50 µL of the Denaturing Solution to each well in the 96-well Low Binding Microplates. Cover the plate with Plate Sealer and incubate at 37°C for 15 minutes on the shaker at 300 rpm.

*Note 1: the incubation temperature should not be less than 37°C.*

*Note 2: After adding the Denaturing Solution, it is common for the reaction solution to become turbid. In cases where the IgG concentration is too high(≥10 mg/mL), extensive antibody precipitation may occur, making it difficult to accurately aspirate the supernatant, leading to reduced recovery rates. It is advisable to briefly centrifuge the solution prior to aspirating the supernatant.*

*Note 3: If you are still experiencing low recovery rates despite carefully following the aforementioned procedure, it may be necessary to dilute your sample further before conducting the assay, using Sample Dilution Buffer. Dilution of samples to a range of* ***≤1 mg/mL*** *is usually sufficient to achieve acceptable recovery.*

**Standards and Samples Incubation**

3. Add 75 µL of the Detection Buffer to each well in the Protein A Capture Plate.
4. Move 25 µL the denatured standard solutions, quality controls and samples from the wells in the 96-well Low Binding Microplates to the wells of the Protein A Capture Plate.
5. Cover the plate with Plate Sealer and incubate at 25°C for 30 minutes on the shaker at 300 rpm.
6. Dump the contents of the wells into waste and tap the inverted plate onto absorbent paper to remove residual liquid.



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7. Wash the plate with 260 µL of 1×Wash Solution five times.
8. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

**Biotin Anti-Protein A Antibody Incubation**

9. Add 100 µL of Biotin Anti-Protein A Antibody to all the testing wells.
10. Cover the plate with Plate Sealer and incubate at 25°C for 30 minutes on the shaker at 300 rpm.
11. Dump the contents of the wells into waste and tap the inverted plate onto absorbent paper to remove residual liquid.
12. Wash the plate with 260 µL of 1×Wash Solution five times.
13. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

**Streptavidin-HRP Incubation**

14. Add 100 µL of Streptavidin-HRP to all the testing wells.
15. Cover the plate with Plate Sealer and incubate at 25°C for 10 minutes on the shaker at 300 rpm.
16. Dump the contents of the wells into waste and tap the inverted plate onto absorbent paper to remove residual liquid.
17. Wash the plate with 260 µL of 1×Wash Solution five times.
18. Tap the inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

**Absorbance Measurement and Calculation**

19. Add 100 µL of TMB Solution to each well and incubate the plate in the dark at 25°C for 15 minutes without shaking(start timing after the addition of TMB Solution to the first well).
20. Add 50 µL of Stop Solution to each well to stop the reaction.
21. Read the absorbance in the microplate reader at 450/650 nm.
22. Plot the standard curve with the Protein A ligand concentration (ng/mL) on the x-axis and the corresponding mean absorbance value (OD450-OD650) on the y-axis.
23. Using a 4- or 5-parameter logistic curve fitting program, calculate the best-fitting linear line through the points of the standard curve except for BL samples.



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## IX. ASSAY PROCEDURE SUMMARY

1. • Prepare 1×Wash Solution, Protein A Standard and Capture Plate.
2. •Add 100 µL of the diluted standard solutions, quality controls and samples into the corresponding wells of the 96-well Low Binding Microplates. Add 50 µL of the Denaturing Solution to the Microplates
   •Incubate at 37°C for 15 minutes on the shaker with 300 rpm.
3. • Add 75 µL of the Detection Buffer into the Protein A Capture Plate.
   •Transfer 25 µL of the denatured standard solutions, quality controls and samples from the 96-well Low Binding Microplates to the Protein A Capture Plate.
4. •Incubate at 25°C for 30 minutes on the shaker with 300 rpm.
   •Wash the plate with 260 µL of 1×Wash Solution per well five times.
5. •Add 100 µL of Biotin conjugated anti-Protein A Antibody to all the testing wells and incubate at 25°C for 30 minutes on the shaker with 300 rpm.
   •Wash the plate with 260 µL of 1×Wash Solution per well five times.
6. • Add 100 µL of Streptavidin-HRP to the well and incubate at 25°C for 10 minutes on the shaker with 300 rpm.
   • Wash the plate with 260 µL of 1xWash Solution per well five times.
7. •Add 100 µL of TMB Solution and incubate the plate in dark at 25°C for 15 minutes without shaking.
8. • Add 50 µL of Stop Solution to each well to stop the reaction
   • Read the plate OD450/OD650 immediately.



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## X. ANALYTICAL PERFORMANCE

- **Suggested Calculation of Data**

  Statistical software can be used to create the standard curves. Choose a method with high Goodness of Fit (R^2) to analyze the data, such as a four-parameter logistic (4-PL) model that provides point-to-point curve fitting.

  The standard curve is for demonstration purposes only. It should be prepared each time an assay is performed.

**Table 4. A typical sample data for MabSelect™ PrismA standard curve**

| MabSelect PrismA (ng/mL) | Absorbance (OD 450-OD650 ) | | | MabSelect PrismA (ng/mL) | CV | Recovery Rate |
|---|---|---|---|---|---|---|
| | Duplicate 1 | Duplicate 2 | Average | | | |
| 4 | 3.100 | 3.019 | 3.060 | 3.997 | 1.88% | 100% |
| 2 | 1.678 | 1.738 | 1.708 | 2.013 | 2.48% | 101% |
| 1 | 0.861 | 0.878 | 0.869 | 0.981 | 1.38% | 98% |
| 0.5 | 0.453 | 0.449 | 0.451 | 0.501 | 0.67% | 100% |
| 0.25 | 0.246 | 0.240 | 0.243 | 0.265 | 1.57% | 106% |
| 0.125 | 0.129 | 0.122 | 0.125 | 0.130 | 4.01% | 104% |
| 0.0625 | 0.069 | 0.067 | 0.068 | 0.061 | 1.46% | 98% |
| 0 | 0.014 | 0.012 | 0.013 | / | 8.07% | N/A |

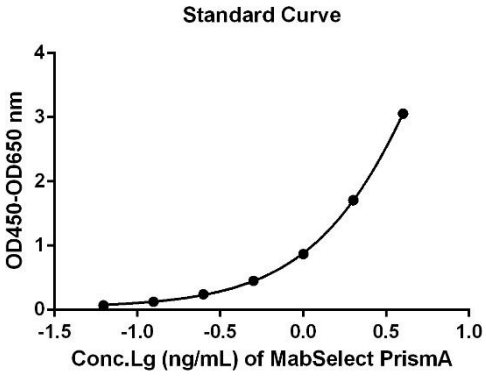


**Figure 1: MabSelect PrismA standard curve**

- **Specificity/Cross-Reactivity**

  This kit is designed to detect native Protein A, recombinant Protein A variants, and alkali-tolerant recombinant Protein A variants. Six different commercially available Protein A variants were tested, and the typical assay working range is shown in **Table 5** and **Figure 2**. Although the kit is not validated for other Protein A variants, this does not mean that they cannot be detected. The user can evaluate the applicability of the assay to a new Protein A variant according to the protocol of the kit.



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**Table 5. Typical assay working ranges for different sources of Protein A ligand**

| Protein A ligand standard | Typical assay working ranges (ng/mL) |
|---|---|
| MabSelect PrismA (Cytiva) | 0.0625-4 ng/ml |
| AT Protein A (Bestchrom) | 0.0625-4 ng/ml |
| MabSelect SuRe (Cytiva) | 0.1-6.4 ng/ml |
| UniMab 50HC Protein A (NanoMicro) | 0.2-12.8 ng/ml |
| MabPurix Protein A (Srpax） | 0.05-3.2 ng/ml |
| rSPA (Repligen) | 0.1-6.4 ng/ml |

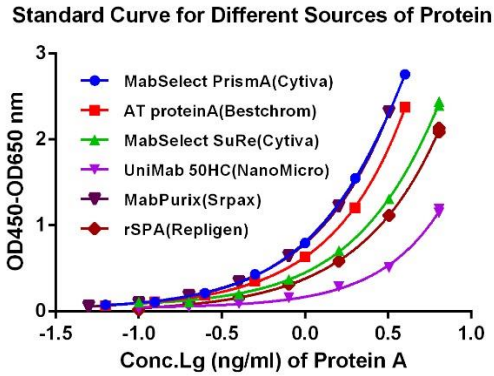


**Figure 2: Various Protein A ligand standard curves**

- **Detection Capability**

  According to the evaluation method recommended in ICH-M10[1] and CLSI EP05-A3[2], the LLOQ for MabSelect PrismA of this kit is 0.0625 ng/mL, and the ULOQ for MabSelect PrismA of this kit is 4 ng/mL.

  *Note: Lower Limit of Quantification (LLOQ) is the lowest amount of an analyte in a sample that can be used for quantification. Upper Limit of Quantification (ULOQ) is the highest amount of an analyte in a sample that can still be used for quantification.*

- **Precision**

  Intra-assay and inter-assay precision were measured in 3 different concentrations (P1 at 3 ng/mL, P2 at 0.5 ng/mL, and P3 at 0.1875 ng/mL) of MabSelect PrismA samples, using 3 batches of kits.

**Table 6. Intra-assay and inter-assay precision of the kit**

| Quality Control | MabSelect PrismA (ng/mL) | Intra-assay (n=10) | | | Inter-assay (n=30) | | |
|---|---|---|---|---|---|---|---|
| | | Measured MabSelect PrismA (ng/mL) | CV | Recovery Rate | Measured MabSelect PrismA (ng/mL) | CV | Recovery Rate |
| P1 | 3 | 2.795 | 3.78% | 93% | 2.794 | 4.21% | 93% |
| P2 | 0.5 | 0.457 | 4.08% | 91% | 0.459 | 4.84% | 92% |
| P3 | 0.1875 | 0.173 | 5.75% | 92% | 0.179 | 11.41% | 95% |



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- **Dilutional Linearity and Hook Effect**

  Samples with different concentrations of MabSelect PrismA were used for the analysis of hook effect (**Table 7**) and dilutional linearity (**Table 8**). The hook effect was not observed in the assayed range.

**Table 7. Hook effect analysis of the kit**

| MabSelect PrismA (ng/mL) | Absorbance (OD450-OD650) | | | CV |
|---|---|---|---|---|
| | Duplicate 1 | Duplicate 2 | Average | |
| 50000 | 5.911 | 5.458 | 5.685 | 5.63% |
| 6250 | 5.795 | 5.485 | 5.640 | 3.89% |
| 781 | 5.909 | 5.905 | 5.907 | 0.05% |
| 100 | 5.903 | 5.778 | 5.840 | 1.51% |
| 10 | 4.655 | 4.898 | 4.777 | 3.60% |
| 5 | 3.025 | 3.035 | 3.030 | 0.23% |

**Table 8. Dilutional linearity analysis of the kit**

| Sample | Dilution Factor | Expected MabSelect PrismA (ng/mL) | Measured MabSelect PrismA (ng/mL) | CV | Recovery Rate |
|---|---|---|---|---|---|
| DL1 | 100 | 1.00 | 0.971 | 1.88% | 97% |
| DL2 | 400 | 0.25 | 0.258 | 1.47% | 103% |
| DL3 | 200 | 0.25 | 0.257 | 2.58% | 103% |
| DL4 | 40 | 0.25 | 0.237 | 1.61% | 95% |
| DL5 | 20 | 0.25 | 0.236 | 0.16% | 94% |

- **Spike & Recovery**

  In some cases, high levels of the human IgGs have been observed to hinder the binding capacity of anti-Protein A antibodies to Protein A, potentially leading to the under-recovery of actual Protein A levels. To investigate the impact of IgG on the accuracy of this kit, different concentrations of Protein A were spiked to 1 or 10 mg/mL Human IgG, and the concentration of Protein A was measured and calculated to determine the recovery rate.

**Table 9. Recovery rate of the kit in human IgG**

| MabSelect PrismA (ng/mL) | Spiked to 1 mg/mL human IgG | | Spiked to 10 mg/mL human IgG | |
|---|---|---|---|---|
| | Measured MabSelect PrismA (ng/mL) | Recovery Rate | Measured MabSelect PrismA (ng/mL) | Recovery Rate |
| 3 | 2.795 | 93% | 2.816 | 94% |
| 0.5 | 0.463 | 93% | 0.453 | 91% |
| 0.1875 | 0.184 | 98% | 0.174 | 93% |



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## XI. TROUBLESHOOTING

| Problem | Probable Cause | Solution |
|---|---|---|
| **Poor Precision** | Wells are not washed or aspirated properly. | Make sure the washing apparatus works properly and wells are dry after aspiration. |
| | Wells are scratched with pipette tips or washing needles. | Dispense and aspirate solution into and out of wells with caution. |
| | Particulates are found in the samples. | Remove any particulates by centrifugation prior to the assay. |
| **Weak/No Signal** | Substrate is not added or added at the wrong time. | Follow the manual to add the substrate properly. |
| | Components are used from other lots or sources. | Use only lot-specific components. |
| | Substrate is contaminated. | Use a new substrate from the same lot. |
| | Volumes of reagents are not correct. | Repeat the assay with the required volumes as noted in the manual. |
| | Plate is not incubated for proper time or temperature. | Follow the manual to repeat the assay. |
| | Plate is not read within the specified time range. | Read the plate within 5 minutes. |
| **High Background** | Plate is not washed properly. | Make sure the washing apparatus works properly. |
| | Substrate is contaminated. | Use new substrate from the same Lot. |
| | Evaporation of wells during incubations. | Perform incubation steps with a plate sealer in a repeat assay. |
| | Incorrect incubation times and/or temperatures. | Follow the manual to repeat the assay. |



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## XII. REFERENCES


1. ICH HARMONISED GUIDELINE: BIOANALYTICAL METHOD VALIDATION AND STUDY SAMPLE ANALYSIS M10
2. CLSI EP05-A3 (Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition)


**For research use only. Not intended for human or animal clinical trials, therapeutic or diagnostic use.**


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