# Troubleshooting

## Poor Precision:

### Possible Source

- Inadequate washing/aspiration of wells
- Inadequate mixing of reagents
- Imprecise/inaccurate pipetting
- Incomplete sealing of plate

### Corrective Action

- Check function of washing system
- Ensure adequate mixing
- Check/calibrate pipettes
- Ensure complete seal on plate

## Poor Standard Complex Curve:

### Possible Source

- Improper standard/In Vivo Capture Antibody handling or dilution
- Incomplete washing/aspiration of wells
- Imprecise/inaccurate pipetting
- Improper buffer/diluent used

### Corrective Action

- Ensure correct preparation and storage of the standard and the In Vivo Capture Antibody
- Check function of washing system
- Check/calibrate pipettes
- Check buffer/diluent preparation, pH

## Low Absorbances:

### Possible Source

- Inadequate reagent volumes added to wells
- Incorrect incubation times/temperature
- Incorrect antibody titration
- Improper buffer/diluent used

### Corrective Action

- Check/calibrate pipettes
- Ensure sufficient incubation times/reagents warmed to RT
- Check Coating Antibody and Standard Complex preparation
- Check buffer/diluent preparation (e.g., for pH, presence of particulates)

- Overly high wash/aspiration pressure from wash system

### Corrective Action

- Utilize manual washing or an automated ELISA plate-washer with the proper pressure settings

## Warnings and Precautions

1. Reagents that contain preservatives may be toxic if ingested, inhaled, or in contact with skin.
2. Handle all serum and plasma specimens in accordance with NCCLS (formerly, National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood-borne infections.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. The Coating Antibody Solution contains < 0.09 % sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

## References

Additional Materials Required But Not Provided

- 96-well Nunc-Immuno™ polystyrene MaxiSorp ELISA flat bottom plates (ThermoFisher Scientific Cat. No. 442404) are recommended
- Plate Sealers with adhesive backing
- ELISA plate reader capable of measuring light absorbance at a 450 nm wavelength
- Single- and Multi-channel pipettes to deliver 50 µl, 100 µl, 200 µl or 300 µl volumes
- Graduated 1 ml, 5 ml, 10 ml, 25 ml pipettes for reagent preparation
- Deionized or distilled water
- Wash bottle or automated ELISA plate washer
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard dilutions
- Absorptometer
- Laboratory timer

Antibody Injection, Serum Collection and Handling

Inject mice intravenously or intraperitoneally with 10 µg of NA/LE™ Biotin Rat Anti-Mouse IL-4 antibody in 200 µl of sterile, endotoxin-free PBS. It is necessary to inject an antibody preparation that lacks preservatives, such as sodium azide, and that is low in endotoxin. Collect blood samples from mice (e.g., in polypropylene microcentrifuge tubes) 2–72 hours post injection and prepare sera. Allow blood samples to clot for 30–60 minutes (4°C). Centrifuge the samples for 10 minutes (4°C) at 1000 X g. Remove serum and assay immediately or store samples at ≤ –70°C. Avoid repeated freeze-thaw cycles. Serum samples should be clear, non-hemolyzed, and non-necropic.

ELISA Plate Coating

1. Dilute the purified Rat Anti-Mouse IL-4 antibody to 2 µg/ml in Coating Buffer. Add 50 µl of the diluted antibody to the wells of an enhanced protein-binding ELISA plate (such as ThermoFisher Scientific Cat. No. 442404).
2. Seal the plate to prevent evaporation. Incubate overnight at 4°C.
3. Bring the plate to room temperature, remove the coating antibody solution, and block non-specific binding by adding 200 µl of Blocking Buffer per well.
4. Seal the plate and incubate at room temperature for 0.5–1 hour.
5. Wash ≥ 3 times with Wash Buffer

Recommended Buffers and Solutions

Note: Do not use sodium azide in these preparations. Sodium azide inactivates the horseradish peroxidase enzyme.

1. Coating Buffer 0.1 M Sodium Bicarbonate/Sodium Carbonate buffer, pH 9.5. Freshly prepare or use within 7 days of preparation, store at 2-8°C.
2. Assay Diluent / Blocking Buffer Prepare 10% fetal bovine serum (FBS), 10% newborn calf serum (NBCS) or 1% bovine serum albumin (BSA; immunomaxx grade) in Phosphate Buffered Saline containing 0.05% Tween-20 (PBS) at pH 7.2–7.4. This buffer should be filtered to remove particulates before use. Freshly prepare or use within several days of preparation, store at 2–8°C.

Note: The BD Pharmingen Assay Diluent (Cat. No. 555213) is recommended.
3. Wash Buffer PBS with 0.05% Tween-20. Freshly prepare or use within 3 days of preparation store at 2–8°C.
4. Substrate Solution Tetramethylbenzidine (TMB) and Hydrogen Peroxide. Note: The BD Pharmingen TMB Substrate Reagent Set (Cat. No. 555324) is recommended for the assay, as it contains 20-plate’s worth of the common ELISA reagents, including all of the buffers and solutions mentioned above.
5. Stop Solution 1 M H3PO4 (Phosphoric Acid) or 1 M H2SO4 (Sulfuric Acid) Note: The BD Pharmingen OptEIA Reagent Set B (Cat. No. 555334) is recommended for the assay, as it contains 20-plate’s worth of the common ELISA reagents, including all of the buffers and solutions mentioned above.

Storage Information

1. Store unopened reagents at 2–8°C. Do not use reagents after expiration date or if precipitation or turbidity is evident.
2. Before use, bring all reagents to room temperature (18–25°C).
3. Lyophilized standards are stable until expiration date. See below for reconstituted standard storage information. After reconstitution, use freshly reconstituted standard within several hours (stored at 4°C).

Limitations of the Procedure

- Samples that generate absorbance values higher than the standard curve should be diluted with Assay Diluent and re-assayed.
- Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
- This In Vivo Capture Assay Set for Mouse Cytokine is intended for use as an integral unit. Do not mix reagents from different lots. Reagents from other manufacturers/other available clones should not be used in this Set.

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