TECHNICAL MANUAL

Mouse EPO (Erythropoietin) Superset Max DIY ELISA

- **SKU CODE:** AEES05403
- **DETECTION PRINCINPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 02/2026

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Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Reactivity:

Mouse

Sample Type:

Serum, Plasma

Range:

7.81-500 pg/mL

Sensitivity:

-

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components as described in section 4. Date of expiration is on the ELISA Box label.

3. Product Description

The Mouse EPO (Erythropoietin) Superset Max DIY ELISA provides all necessary reagents for the development of a high-performance sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for quantitative measurement of Mouse EPO. The kit comprises the following key components: an unconjugated capture antibody, a recombinant protein standard, a biotinylated detection antibody, and a horseradish peroxidase (HRP) conjugate. All included reagents have undergone rigorous functional validation, confirming their compatibility and efficacy within the specified ELISA protocol using the provided standard. This technical guide offers further recommendations and insights to facilitate optimal assay design and performance by the user.

4. Kit Contents

No	Component Name	Specifications	Dilutions	Storage
1	Mouse EPO Micro ELISA pre-Plate	96T*5: 5 plates, 96T | 96T*15: 15plates, 96T	-	-20℃, 12 months
2	Mouse EPO Capture Ab	96T*5: 1 vial, 120µL | 96T*15: 1 vial, 350µL	1/500-1/1000	-20℃, 12 months
3	Mouse EPO Biotinylated Detection Ab	96T*5: 1 vial, 120µL | 96T*15: 1 vial, 350µL	1/500-1/1000	-20℃, 12 months
4	Mouse EPO Reference Standard	96T*5: 5 vials | 96T*15: 15 vials	500 pg/vial	-20℃, 12 months
5	Mouse EPO HRP Conjugate	96T*5: 1 vial, 120µL | 96T*15: 1 vial, 350µL	1/500-1/1000	-20℃(Protect from light), 12 months
6	Technical Manual	1 copy	-	-20℃(Protect from light), 12 months
7	Certificate of Analysis	1 copy	-	-20℃(Protect from light), 12 months
8	Bradford Reagent	96T: 1 vial | 48T: 1 vial	-	4℃

5. Materials & reagents not provided

A. Included in our Ancillary Pack (SKU: AEES03043)

- ELISA Plate Coating Buffer (5X)
- ELISA Plate Blocking Buffer
- Wash Buffer for Sandwich-ELISA (25X)
- Stop Solution (5X)
- HRP-conjugate Diluent
- Biotinylated Antibody Diluent
- Sample Diluent
- One-component TMB Substrate

B. Buffers & Diluents

Note: *The following formula only contains the basic component information of each reagent, which can be optimized according to the experimental requirements and results.*

- Coating Buffer: 1xCBS
- Blocking Buffer: 1xPBS, Protective substance
- Wash Buffer: 3% Tris
- Standard & Sample Diluent: 1xPBS, Protective substance
- Antibody & HRP conjugate Diluent: 1xPBS, Protective substance
- Stop Solution: 5% sulfuric acid

C. Instruments and materials

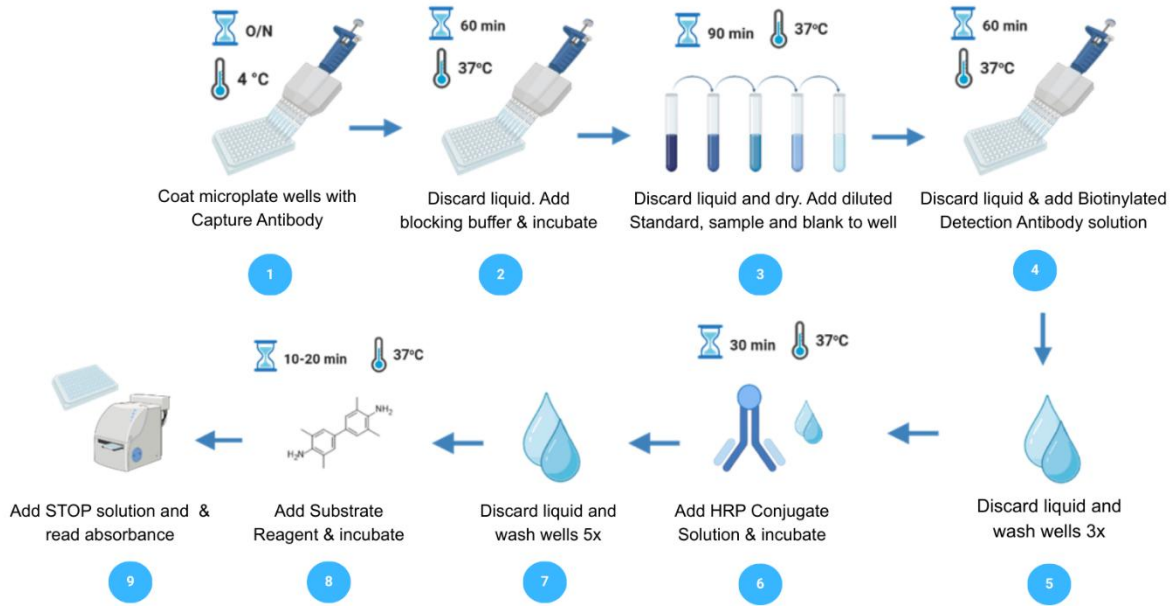
- 96-well high binding ELISA plate
- Ultrapure water
- Precision pipettors, with disposable plastic tips

- Polypropylene or polyethylene tubes to prepare standard, samples, and working solutions. ***Note:*** *Do not use polystyrene, polycarbonate or glass tubes. They bind protein and antibodies.*
- Containers to prepare buffers
- A wash bottle or an automated 96-well plate washer
- Disposable reagent reservoirs
- Plate sealers/adhesive plate covers
- A standard microtiter plate reader for measuring absorbance at 450 nm

6. Precautions

1. This kit is ideal for research purposes only and not for diagnostics or therapeutic uses.
2. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
3. Allow all reagents and samples to reach room temperature before use.
4. Ensure unopened and unused plate are kept dry to avoid contamination.
5. Before using the kit, centrifuge tubes to spin down standard and/or antibody.
6. Prepare all reagents, samples and standards as directed in this manual.
7. Duplicate wells are recommended for both standard and sample testing.
8. Do not let the microplate wells dry during the assay.
9. Maintain consistent incubation times and temperatures as variations can affect results.
10. Do not reuse tips and tubes to avoid cross contamination.
11. Do not use reagents beyond the expiration date of the kit.

7. Assay Summary



8. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions**:
    - **Short-term**: 2-8 °C for up to 5 days.

- **Medium-term**: -20 °C for up to 6 months.
- **Long-term**: -80 °C or cryopreservation in liquid nitrogen.

- **Thawing Protocol**: Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles**: Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

A. Blood-Derived Samples

- **Serum:** Allow whole blood to coagulate at room temperature (2 h) or 2-8 °C overnight. Centrifuge at 1000 × g for 20 min and collect the supernatant. Store or use immediately.
- **Plasma:** Collect in anticoagulant tubes (EDTA, citrate, or heparin), mix gently, and centrifuge within 30 min at 1000 × g, 2-8 °C for 15 min. Store or assay as needed.

B. Tissue Homogenates

Tissue samples should be homogenized prior to use. Avoid buffers containing NP-40, Triton X-100, or DTT, as these strongly inhibit the assay. We recommend using 50 mM Tris + 0.9% NaCl + 0.1% SDS, pH 7.3.

The recommended protocol is as follows:

- **Sample Collection and Washing**
  - Place the target tissue on ice.
  - Rinse the tissue with pre-cooled PBS buffer (0.01 M, pH 7.4) to remove residual blood.
  - Weigh the tissue for further processing.
- **Homogenization**
  - Homogenize the tissue on ice using an appropriate lysis buffer.
  - The lysate volume should correspond to the tissue weight; typically, 9 mL PBS is used per 1 g of tissue. It is recommended to add protease inhibitors

to the PBS (e.g., 1 mM PMSF). ***Note:*** *PBS buffer or mild RIPA lysis buffer can be used for homogenization. When using RIPA, adjust pH to 7.3.*

- **Cell Disruption**
  - Further disrupt the tissue using ultrasonic homogenization or freeze–thaw cycles.
    - Ultrasonic homogenization: Keep samples on an ice bath during sonication to avoid overheating.
    - Freeze–thaw cycles*:* Repeat twice for effective lysis.
- **Centrifugation and Storage**
  - Centrifuge the homogenate at 5000 × g for 5 minutes.
  - Collect the supernatant for immediate analysis, or aliquot and store at –20°C or –80°C for future assays.
- **Protein Concentration Measurement**
  - Determine total protein concentration using Bradford Reagent included in this kit.
  - For ELISA assays, the total protein concentration should generally be 1–3 mg/mL.
  - Tissues with high endogenous peroxidase levels (e.g., liver, kidney, pancreas) may react with TMB substrate, causing false positives. If this occurs, treat samples with 1% H_2O_2 for 15 minutes before repeating the assay.

Note: *Liver, kidney, and pancreas samples often contain high levels of endogenous peroxidase, which may react with the chromogenic substrate at elevated sample concentrations, potentially resulting in false positive signals.*

If analysis of these tissues is required, a gradient dilution assay is recommended. A proportional decrease in signal with increasing dilution typically indicates minimal interference and supports the accuracy of the results.

To further minimise potential interference, samples can be pre-treated with 1% hydrogen peroxide (H_2O_2) for 15 minutes prior to testing. To prepare the treatment solution, add 1 µl of pure H_2O_2 to 100 µl of sample (1% v/v).

C. Cell Culture Supernatant

Centrifuge the sample at 2500 rpm for 5 minutes at 2–8°C. Carefully collect the clarified cell culture supernatant for immediate analysis, or aliquot and store it at –80°C for future assays.

D. Cell Lysates

- **Suspension Cell Lysate:** Centrifuge the cell suspension at 2500 rpm for 5 minutes at 2–8°C and collect the cell pellet. Wash the pellet with pre-cooled PBS (0.01 M, pH 7.4) and mix gently. Repeat centrifugation and discard the supernatant. Add 0.5–1 mL of cell lysis buffer containing an appropriate protease inhibitor (e.g., PMSF, final concentration: 1 mM). Lyse the cells on ice for 30–60 minutes or disrupt them using ultrasonic homogenization.
- **Adherent Cell Lysate:** Remove the supernatant and wash the cells three times with pre-cooled PBS. Add 0.5–1 mL of cell lysis buffer supplemented with an appropriate protease inhibitor (e.g., PMSF at a final concentration of 1 mmol/L). Scrape the adherent cells using a cell scraper and transfer the cell suspension to a centrifuge tube. Lyse the cells on ice for 30–60 minutes or disrupt the cells by ultrasonic treatment.

Follow next steps for protein extraction and supernatant collection:

- **Protein Release and DNA Disruption**
    - During lysis, pipette gently or intermittently shake the tube to enhance protein extraction.
    - Mucilaginous material formed during lysis is DNA, which can be broken down by ultrasonic disruption (3–5 mm probe, 150–300 W, 3–5 seconds per cycle, with 30-second intervals for 1–2 minutes total).

- **Supernatant collection**
    - After lysis or ultrasonic treatment, centrifuge the lysate at 10,000 rpm for 10 minutes at 2–8°C. Collect the supernatant for immediate use or aliquot and store at –80°C for future assays.

Notes: *Refer to the "Tissue Sample Notes" for additional buffer and inhibitor recommendations.*

E. Other Sample Types

For more information about how to process other sample types, (e.g., body fluids, breast milk & more), plea se contact our Tech Support Team at techsupport@assaygenie.com.

8.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit https://www.assaygenie.com/bradford-protein-assay-protocol/ to view the full protocol.

9. Reagent Preparation

A. Equilibrate Reagents

Bring all reagents to room temperature (18–25°C) before use. If the entire kit will not be used in a single assay, only remove the necessary strips and reagents for the current experiment. Store the remaining components under the recommended conditions.

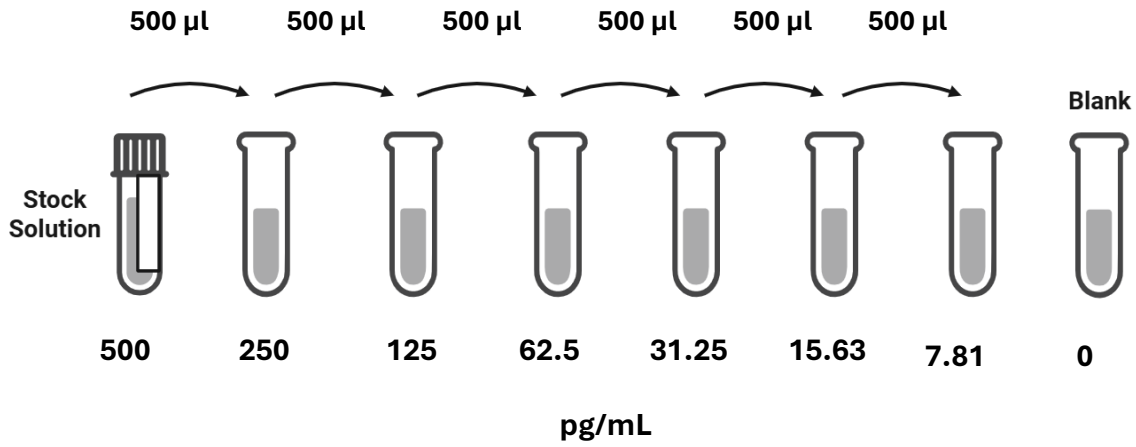
B. Plate Coating

1. Dilute the capture antibody to the working concentration using ELISA Plate Coating Buffer (1x). A dilution of 1:500 to 1:1000 is recommended.
2. Remove the Micro ELISA pre-plate from its packaging. Add 100 µL of the diluted capture antibody to each well. Cover the plate with the sealing film provided and incubate overnight at 2–8°C.

3. Decant the liquid from each well without washing. Add 200 µL of ELISA Plate Blocking Buffer to each well. Cover with the sealer and incubate for 1 hour at 37°C.
4. After incubation, decant the blocking buffer, do not wash. The plate is now ready for sample addition. Alternatively, the plate can be dried at 37°C for 30 minutes and stored at -20°C for up to 6 months when sealed with a desiccant.

C. Standard Working solution

1. Centrifuge the standard at 10,000 × g for 1 minute. Add 1 mL of Sample Diluent, let it stand for 10 minutes, and gently invert several times. Once fully dissolved, mix thoroughly using a pipette. This will yield a working solution with a concentration of **500** pg/mL. ***Alternatively:*** *Add 1 mL of Sample Diluent, let it stand for 1–2 minutes, then mix gently using a low-speed vortex mixer. Any bubbles formed during vortexing can be removed by brief low-speed centrifugation*. Proceed to prepare serial dilutions as required. The recommended dilution series is: **500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 0** pg/mL**.**
2. Prepare 7 Eppendorf tubes and add 500 µL of Sample Diluent to each. Transfer 500 µL of the **500** pg/mL working solution into the second tube and mix well to create the **250** pg/mL solution. Continue serial two-fold dilutions by transferring 500 µL from one tube to the next, mixing thoroughly at each step. ***Note:*** *Do not add any solution to the final tube, which serves as the blank. Prepare the gradient-diluted standards fresh before use.*



D. Biotinylated Detection Ab working solution

1. Calculate the required volume in advance based on 100 µL per well. Prepare slightly more than the calculated amount to account for pipetting losses.
2. Centrifuge the concentrated Biotinylated Detection Antibody at 800 x g for 1 minute. Dilute it to the working concentration using Biotinylated Antibody Diluent. A dilution of 1:500 to 1:1000 is recommended.
3. Prepare the working solution fresh immediately before use.

E. HRP Conjugate working solution

1. Calculate the required volume in advance based on 100 µL per well. Prepare slightly more than the calculated amount to compensate for pipetting losses.
2. Centrifuge the HRP Conjugate at 800 x g for 1 minute. Dilute to the working concentration using HRP Conjugate Diluent. A dilution of 1:500 to 1:1000 is recommended.
3. Prepare the working solution fresh immediately before use.

F. Wash Buffer

1. Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of working Wash Buffer. ***Note:*** *If crystals are present in the concentrate, warm the solution in a 40°C water bath and gently mix until the crystals are fully dissolved. Prepare fresh and use on the same day.*

10. Assay Procedure

1. **Sample, Blank and Standard Addition**: Determine the wells for the diluted standards, blank, and samples. Add 100 µL of each dilution of standard, blank, and sample into the designated wells. ***Note:*** *It is strongly recommended to assay all standards and samples in duplicate. The appropriate sample dilution ratio should be determined through preliminary experiments or by consulting technical support*.
2. **First Incubation:** Cover the plate with the sealing film provided and incubate for 90 minutes at 37°C. ***Important:*** *Add all solutions carefully to the bottom of each well, avoiding contact with the inner wall and minimizing foaming*.
3. **Biotinylated Detection Antibody Addition:** Decant the liquid from each well without washing. Immediately add 100 µL of Biotinylated Detection Antibody working solution to each well.
4. **Second Incubation:** Cover with a new sealer and incubate for 1 hour at 37°C.
5. **Washing Step (3x)**: Decant the solution from each well. Add 350 µL of Wash Buffer to each well, let it soak for 1 minute, then aspirate or decant the liquid and gently blot dry on clean absorbent paper. Repeat this washing step three times. ***Tip:*** *A microplate washer can be used for this and subsequent wash steps. Do not allow the wells to dry after washing. Proceed immediately to the next step.*
6. **HRP Conjugate Addition**: Add 100 µL of HRP Conjugate working solution to each well.
7. **Third Incubation:** Cover with a new sealer and incubate for 30 minutes at 37°C.
8. **Washing Step (5x):** Decant the solution and repeat the washing process as described in Step 5, this time five times.
9. **Substrate Reaction and Incubation:** Add 90 µL of Substrate Reagent to each well. Cover the plate and incubate for approximately 15 minutes at 37°C, protecting it from light. ***Note:*** *Incubation time can be adjusted based on colour development but should not exceed 30 minutes. Preheat the microplate reader for approximately 15 minutes before* reading.
10. **Stop Reaction:** Add 50 µL of Stop Solution to each well. ***Important****: Add the Stop*

Solution in the same order as the Substrate Reagent to maintain consistency.

11. **OD Measurement:** Immediately measure the optical density (OD) of each well at 450 nm using a microplate reader.

11. Data Analysis

Average the duplicate readings for each standard, control, and sample, then subtract the mean optical density of the zero standard. Construct a standard curve by plotting Mouse EPO concentration on the y-axis against absorbance on the x-axis and fit the data using an appropriate best-fit curve.

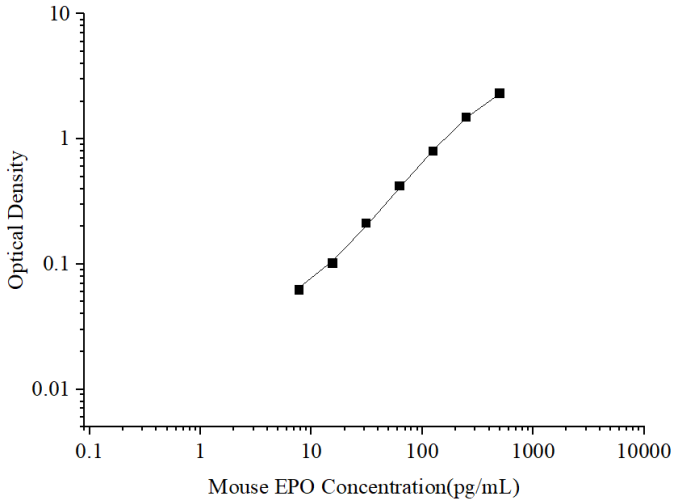
For diluted samples, multiply the concentration obtained from the standard curve by the corresponding dilution factor to determine the final concentration. Data analysis and curve fitting may be performed using suitable plotting software (e.g., CurveExpert).

Note: *If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution*.

12. Typical Data

Standard Curve

Concentration (pg/mL)	OD	Corrected OD
500	2.322	2.229
250	1.579	1.486
125	1.018	0.925
62.5	0.582	0.489
31.25	0.330	0.237
15.63	0.197	0.104
7.81	0.150	0.057
0	0.093	-



Specificity

This kit recognizes Mouse EPO in samples. No significant cross-reactivity or interference between Mouse EPO and analogues was observed.

13. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution.	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.