Equine EPO Do-It-Yourself ELISA

Catalog Number: DIY1146E-003

Storage: 2-8ºC

Stability: 12 months (from date of receipt)

Country of Origin: USA

Description: Contains capture antibody, standard, and detection antibody for development of an ELISA. The antibodies have been determined to function in an ELISA with the standard provided. Optimal buffers, concentrations, incubation times, incubation temperatures, and methods for the ELISA have not been determined. **A working knowledge of ELISA is strongly recommended.** The quantities of components provided are not matched. Components may also be purchased separately.

Included Components:

Description	Usage	Quantity	Catalog Number
Anti-Equine EPO Polyclonal Antibody	Capture Antibody	100 µg	KP1144E-100
Recombinant Equine EPO	Standard	5 µg	RP0932E-005
Biotinylated Anti-Equine EPO Polyclonal Antibody	Detection Antibody	50 µg	KPB1145E-050

Suggested Reagents:

Reagent	Suggested Formulation
DPBS	0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4
96-well ELISA Plate	Clear, flat-bottom, high-binding 96-well plate, 8-wells per strip, 350 µL per well *ELISA Plates: Catalog # AR0658-005*
Standard and Sample Diluent	The optimal Standard and Sample Diluent will need to be determined for each sample type to obtain optimal recovery and linearity. The appropriate Standard and Sample Diluent will mimic the sample's response to a known quantity of protein standard and will provide linear results when diluted. Often a 1:4 dilution of the sample in Reagent Diluent will provide acceptable recovery and linearity.
Reagent Diluent and Blocking Buffer	4% BSA in DPBS, 0.2 µm filtered
Wash Buffer	0.05% Tween®-20 in DPBS
Streptavidin-HRP	Enzymatic reagent to react with biotinylated detection antibody *Streptavidin-HRP: Catalog # AR0068-001*
Substrate	3,3',5,5'-tetramethylbenzidine (TMB) Substrate *ELISA Accessory Pack: Catalog # AR0133-002*
Stop Solution	0.18 M Sulfuric Acid *ELISA Accessory Pack: Catalog # AR0133-002*
Plate Sealer	Adhesive film to prevent evaporation

REAGENTS FOR ANIMAL MODEL AND VETERINARY RESEARCH

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Do-It-Yourself ELISA

Generic ELISA Protocol: **Optimal buffers, concentrations, incubation times, incubation temperatures, and methods have not been determined. A working knowledge of ELISA is strongly recommended.**

1. Prepare Capture Antibody in DPBS at desired working concentration.
2. Add 100 µL of Capture Antibody Working Solution to appropriate wells.
3. Cover plate with Plate Sealer and incubate at room temperature (20-25°C) for 12-24 hours.
4. Empty Capture Antibody Working Solution from plate. Blot plate onto paper towels or other absorbent material.
5. Add 100 µL of Blocking Buffer to appropriate wells.
6. Cover plate with Plate Sealer and incubate at room temperature for 1-3 hours.
7. Empty Blocking Buffer from plate. Blot plate onto paper towels or other absorbent material.
8. Prepare Standard and sample as desired with Standard and Sample Diluent.
9. Add 100 µL of Standard or sample to appropriate wells.

   Note: Run each Standard or sample in duplicate.
10. Cover plate with Plate Sealer and incubate at room temperature for 1 hour.
11. Wash plate FOUR times with Wash Buffer.

    Note: Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.
12. Prepare Detection Antibody in Reagent Diluent at desired working concentration.
13. Add 100 µL of Detection Antibody Working Solution to each well.
14. Cover plate with Plate Sealer and incubate at room temperature for 1 hour.
15. Wash plate FOUR times with Wash Buffer as described in step 11.
16. Prepare Streptavidin-HRP in Reagent Diluent at desired working concentration.
17. Add 100 µL of Streptavidin-HRP Working Solution to each well.
18. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes.
19. Wash plate FOUR times with Wash Buffer as described in step 11.
20. Add 100 µL of TMB Substrate Solution to each well.
21. Develop the plate in the dark at room temperature for 30 minutes or as desired.

    Note: Do **NOT** cover plate with Plate Sealer.
22. Stop reaction by adding 100 µL of Stop Solution to each well.
23. Measure absorbance on a plate reader at 450 nm.