Human Erythropoietin (EPO) ELISA Kit

(Pre-Aliquoted Standard, Liquid)

Catalog Number：CEA-C070

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human EPO from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

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NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

Manufactured and distributed by
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CONTENTS

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA070-C01	Pre-coated Anti-EPO Antibody Microplate	1 plate
CEA070-C02	Human EPO Standard-200 mIU/mL	500 µL
CEA070-C03	Human EPO Standard-100 mIU/mL	500 µL
CEA070-C04	Human EPO Standard-50 mIU/mL	500 µL
CEA070-C05	Human EPO Standard-25 mIU/mL	500 µL
CEA070-C06	Human EPO Standard-12.5 mIU/mL	500 µL
CEA070-C07	Human EPO Standard-6.25 mIU/mL	500 µL
CEA070-C08	Human EPO Standard-3.125 mIU/mL	500 µL
CEA070-C09	Biotin-Anti-EPO Antibody Con. Solution	100 µL
CEA070-C10	Biotin-Antibody Dilution Buffer	8 mL
CEA070-C11	Streptavidin-HRP Con. Solution	500 uL
CEA070-C12	HRP Dilution Buffer	15 mL
CEA070-C13	20× Washing Buffer	50 mL
CEA070-C14	Sample Dilution Buffer	15 mL ×2
CEA070-C15	Substrate Solution	12 mL
CEA070-C16	Stop Solution	6 mL

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

STORAGE

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.

For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-EPO Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.

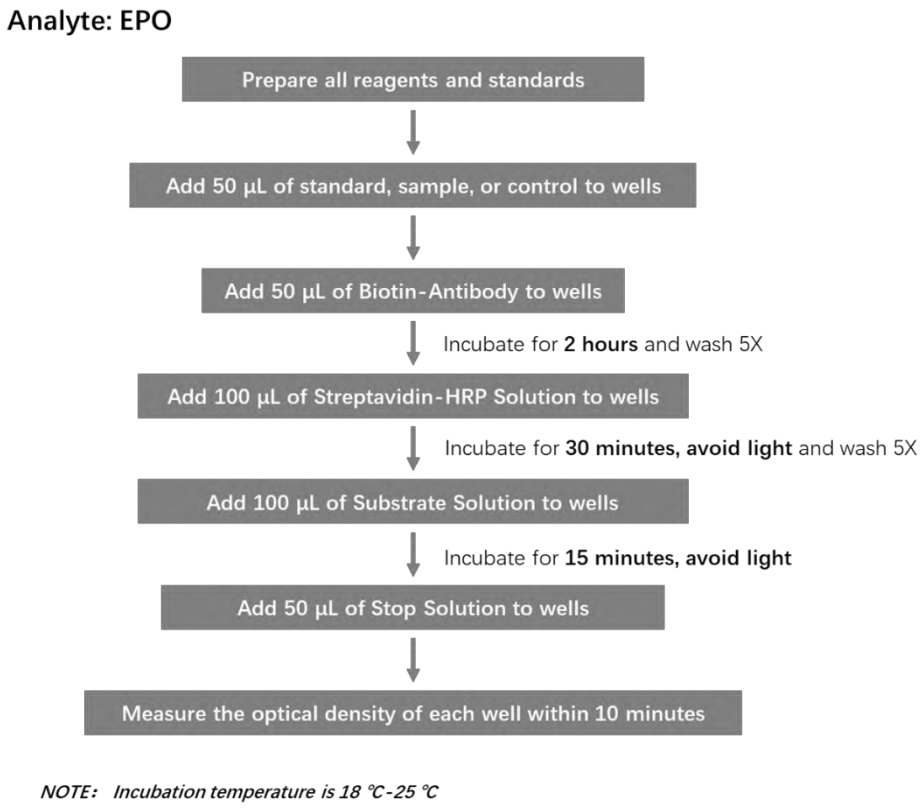
NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

REQUIRED MATERIALS NOT SUPPLIED.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

WORKFLOW



NOTE: Incubation temperature is 18 ℃-25 ℃

PREPARE THE WORKING BUFFERS AND STANDARD DILUTIONS.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin Antibody Solution: Add 60 µL of Biotin-Anti-EPO Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Streptavidin-HRP Solution: Add 240 µL of Streptavidin-HRP Con. Solution to 12 mL of HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the spiking sample (If required)

1. Blank Matrix Spiking
   - Matrix Selection： Prioritize blank matrices homologous to test samples (e.g., serum, plasma, cell supernatant).
   - Concentration Setting: Low concentration = 3-5 × LOD (Limit of Detection), medium concentration = midpoint of the linear range, high concentration = upper limit of the linear range; adjust appropriately to cover routine detection concentrations.
2. Spiking Amount for Test Samples: When spiking test samples, spike at 3 levels (low, medium, high) of the expected sample concentration (e.g., 0.5×, 1×, 2× expected concentration), with final total concentration within the kit's standard curve linear range.
3. Spiking Operation: Prepare using "matrix + standard solution"; spiking volume ≤ 10% of the total matrix volume to avoid excessive matrix dilution, gently mix well.

PROCEDURE OF ASSAY

1. Add 50 µL of EPO Standard, sample, or control to wells.
2. Add 50 µL Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **2 hours.**
3. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
4. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light.**
5. Repeat step 3.
6. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
7. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing. ***Note:*** *the color in the wells should change from blue to yellow.*
8. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes. ***Note:*** *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

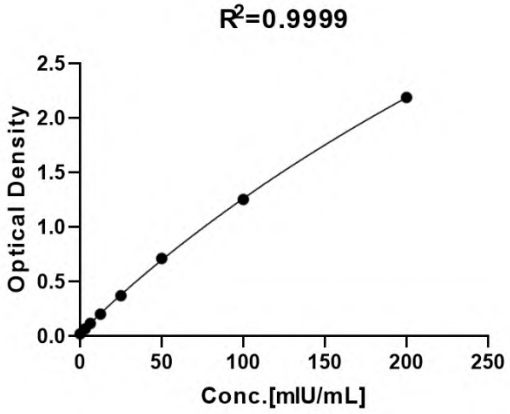
CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample.
2. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

EPO Standard(mIU/mL)	$OD_{450nm-630nm}$
200	2.189
100	1.253
50	0.712
25	0.370
12.5	0.201
6.25	0.116
3.125	0.067
0	0.021



PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of EPO is typically less than 2.0 mIU/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts