AssayGenie

TECHNICAL MANUAL

Rabbit GRO alpha/CXCL1 ELISA Kit (Preliminary)

- **SKU CODE:** SBRS7295
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 09/2026

Rabbit GRO alpha/CXCL1 ELISA Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Detection Method:

Sandwich ELISA (HRP-Streptavidin / TMB, read at 450 nm)

Sample Type:

Serum, plasma, cell culture supernatants

Reactivity:

Rabbit

Range:

To be determined during the development process

Sensitivity:

To be determined during the development process

Storage:

-20°C for up to 1 year from the date of shipment (see section 2)

Expiry:

See Kit Label

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.

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see the table in section 4.

3. Product Description

This Sandwich ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of the target protein in biological samples such as serum, plasma or cell culture supernatants. This assay employs a target-specific capture antibody coated on a 96-well plate. Standards and samples are pipetted into the wells and specific target protein present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated specific detection antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of specific target protein bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

4. Kit Contents

No	Component Name	Size / Description	Storage / Stability After Preparation
1	Target Protein Microplate	96 wells (12 strips x 8 wells) coated with anti-target protein.	1 month at 4°C*
2	Target Protein Standard	2 vials of target protein. 1 vial is enough to run each standard in duplicate.	1 week at -80°C
3	Target Protein Detection Antibody	2 vials of biotinylated anti-target protein. Each vial is enough to assay half the microplate.	5 days at 4°C
4	Wash Buffer	25 ml of 20X concentrated solution.	1 month at 4°C
5	HRP-Streptavidin	200 µl of concentrated HRP-conjugated streptavidin.	Do not store and reuse.
6	TMB One-Step Substrate Reagent	12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
7	Stop Solution	8 ml of 0.2 M sulfuric acid.	N/A
8	Assay Diluent(s)	Diluents for samples, standard and HRP-Streptavidin.	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

5. Precautions

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.
2. Ensure unopened and unused plate is kept dry to avoid contamination.
3. Before using the kit, centrifuge tubes to spin down standard & antibodies.
4. Avoid light for storage of TMB reagents.
5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.
6. Duplicate wells are recommended for both standard and sample testing.
7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.
8. Do not reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

6. Sample Preparation

Note: *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.*

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.

- **Anticoagulant Guidance:** The document provides detailed recommendations on the selection and properties of EDTA, citrate, and heparin for various analytical requirements.

B. Tissue Homogenates

- Tissues should be dissected on ice and washed in cold PBS to remove blood residues.
- Homogenization is performed using buffers such as PBS, Tris/NaCl/SDS, or RIPA (pH 7.3), with protease inhibitors (e.g., PMSF).
- Disruption methods include mechanical homogenization, ultrasonic lysis, or freeze-thaw cycling.
- After processing, centrifuge at 5000 × g for 5 min and collect the supernatant.
- Special consideration is needed for tissues rich in peroxidases (e.g., liver), which may interfere with TMB-based detection.

C. Cell Culture Supernatant and Lysates

- **Supernatant:** Harvest from adherent or suspension cultures (60–90% confluency), centrifuge at 2500 rpm, 2–8 °C for 5 min.
- **Lysates:**
    - Cells are collected and washed with cold PBS.
    - Lysis is performed using RIPA buffer or equivalent formulations with protease inhibitors.
    - Incubate on ice for 30–60 min. Ultrasound may be used to shear DNA and improve lysis efficiency.
    - Centrifuge at 10,000 rpm, 2–8 °C for 10 min. Collect the clarified supernatant.
- **Selection Guidance:** Use supernatant for secreted targets and lysate for intracellular proteins.

D. Other Sample Types

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

7. Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. **Sample dilution:** Samples should be diluted with the provided Assay Diluent(s).

   Note: Levels of target protein may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. **Preparation of standard:** Appropriate standard preparation will be determined during the development process.

	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Standard + ___ µl	___ µl	___ µl	___ µl	___ µl	___ µl	___ µl	___ µl
Conc.	___	___	___	___	___	___	___	0

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
6. Appropriate Detection Antibody preparation will be determined during the development process.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted with Assay Diluent.

8. Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

9. Data Analysis

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

10. Typical Data

Standard Curve

An example of a typical standard curve for this kit will be available after the development process.

Sensitivity

The minimum detectable dose of the target protein will be determined during the development process.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Specificity

Specificity will be determined during the development process.

Recovery

Recovery will be determined during the development process by spiking various levels of the target protein into serum, plasma and cell culture media.

Linearity

Linearity will be determined in serum, plasma and cell culture media during the development process.

Precision

- Intra-Assay: CV<10%
- Inter-Assay: CV<12%

11. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents	Confirm the required reagent added in each step. Also repeat the assay and verify.
	Components used from different kits	Use the component included in the same kit. Also repeat the assay and verify
	Forget to add some reagents	Verify whether the required reagent is added
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Poor standard curve	Inappropriate curve fitting model	Try to plot the curve by different fitting Models.
Samples without signal	The amount of sample is lower than the detection range	Decrease dilution ratio or concentrate the sample.
	The detection target is incompatible with the buffer	Verify the compatibility of sample storage buffer with the sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline.
	Longer storage of sample or freeze- thaw cycle.	Aliquot and store samples according to the assay requirement
High CV%	Precipitate is formed in the well during staining	Increase the dilution ratio of the sample.
	Unclean plate	Don't touch the bottom of the plate during the assay
	Foam is found in the well	Avoid foaming during reading in a microplate Reader.

	Each well is washed unevenly	Check whether the tube of the washer is smooth.
	Reagents are not completely mixed	Mix all reagents completely
	Inconsistent pipetting	Use calibrated pipette and correct pipettingmethod
Poor standard curve	Inaccurate pipetting Improper standard dilution	Check pipettes Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing
Low signal	Improper preparation of standard and/or biotinylated antibody Too brief incubation times Inadequate reagent volumes or improper dilution	Briefly spin down vials before opening. Dissolve the powder thoroughly. Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). Check pipettes and ensure correct preparation
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. If using a plate washer, ensure that all ports are unobstructed. Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit Stop solution	Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light. Add stop solution to each well before reading plate

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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.