



Human ERLIN1(Endoplasmic Reticulum Lipid Raft Associated Protein 1) Microsample ELISA Kit

Cat: ELK6996MS

Detectable Sample Type: Tissue homogenates and other biological fluids.

Sensitivity: 0.107 ng/mL

Detection Range: 0.32-20 ng/mL

Specificity: This assay has high sensitivity and excellent specificity for detection of Human ERLIN1.

No significant cross-reactivity or interference between Human ERLIN1 and analogues was observed.

Please refer to the outer packaging label of the kit for the specific shelf life.

KIT Components

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C
Standard (Lyophilized)	1 vial	2 vials	4°C/-20°C
Biotinylated Antibody (100×)	60 µL	120 µL	4°C/-20°C
Streptavidin-HRP (100×)	60 µL	120 µL	4°C/-20°C
Standard/Sample Diluent Buffer	10 mL	20 mL	4°C/-20°C
Biotinylated Antibody Diluent	6 mL	12 mL	4°C/-20°C
HRP Diluent	6 mL	12 mL	4°C/-20°C
Wash Buffer (25×)	10 mL	20 mL	4°C/-20°C
TMB Substrate Solution	6 mL	10 mL	4°C/-20°C (store in dark)
Stop Reagent	3 mL	6 mL	4°C/-20°C
Plate Covers	1 piece	2 pieces	RT



Special Explanation

1. *If the kit is opened, Store the whole kit at 4°C. If the kit is not used up in 1 week. Store the Pre-Coated Microplate, Standard ,Biotinylated Antibody and Streptavidin-HRP at -20°C, the rest reagents at 4°C, please used up within 6 months.

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3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Test Principle

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Human ERLIN1. Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Human ERLIN1. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Human ERLIN1, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450\text{nm} \pm 10\text{nm}$. The concentration of Human ERLIN1 in the samples is then determined by comparing the OD of the samples to the standard curve.





in aliquots at $\leq -20^{\circ}\text{C}$.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container.

Centrifuge at $2000 \times g$ for 15 minutes at $2-8^{\circ}\text{C}$ to remove particulate matter, assay immediately or aliquot and store at $\leq -$



Summary



1. After the kit is equilibrated at room temperature, add 25 μL of Standard Working Buffer (gradually diluted according to the instructions) or 25 μL



Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use. Make sure all components are dissolved and mixed well before using the kit.
2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.
4. **Standard Working Solution** - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with





avoid contact between TMB Substrate Solution and metal to prevent color development. TMB is contaminated if it turns blue color before use and should be discarded. TMB is toxic, avoid direct contact with hands.

8. Bacterial or fungal contamination of either samples or reagents or cross-contamination, between reagents may cause erroneous results.

Samples Preparation



Cover and incubate for 50 minutes at 37°C.

4. Repeat the aspiration, wash process for total 3 times as conducted in step 2.
5. Add 100 µL of Streptavidin-HRP Working Solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.
6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.
7. Add 90 µL of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.
8. Add 50 µL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.
9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.



Calculation of Results

Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the Human ERLIN1 concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the Standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.



Precision

Intra-assay Precision (Precision within an assay): **CV% < 8%**

Three samples of known concentration were tested twenty times on 1 plate to assess intra-assay precision.

Inter-assay Precision (precision between assays): **CV% < 10%**

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Recovery

Matrices listed below were spiked



Declaration

1. For research use only. Not intended for diagnostic use.
2. The kit may not be suitable for special experimental samples where the validity of the experiment itself is uncertain, such as gene knockout experiments.
3. Certain natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins, may not be detected because they do not match the detection antibody and capture antibody used in this product.
4. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.

**NO color plate**

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN ₃ , etc.), and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN ₃ preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN ₃
	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.



Description of results	Possible reason	Recommendations and precautions
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to



Description of results	Possible reason	Recommendations and precautions
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid