LRRC15 ELISA Kit, Human

【Ⅰ】About this kit

【Ⅰ－1】Background and Measurement Principal

LRRC15 is expressed on stromal fibroblasts in many solid tumors (e.g., breast, head and neck, lung, pancreatic) as well as directly on a subset of cancer cells of mesenchymal origin (e.g., sarcoma, melanoma, glioblastoma). LRRC15 expression is induced by TGFβ on activated fibroblasts (αSMA+) and on mesenchymal stem cells. These collective findings suggested LRRC15 as a novel CAF and mesenchymal marker with utility as a therapeutic target for the treatment of cancers with LRRC15-positive stromal desmoplasia or cancers of mesenchymal origin. [1)]

This product is a two-step sandwich ELISA kit to detect LRRC15 protein using a couple of highly specific monoclonal antibodies against the extracellular domain of human LRRC15.

【Ⅰ－2】Features

・Detects high sensitivity Human LRRC15 by a two-step sandwich method using solid phase anti LRRC15 antibody and HRP conjugated anti LRRC15 antibody.
・The detection limit and quantification limit are 3.0pg/mL and 9.1pg/mL .
・No special equipment is required. Standard microplate reader capable of reading at 450nm will do the job.

【Ⅰ－3】Kit Principle

This ELISA kit uses two-step Sandwich ELISA principle. The ELISA plate provided in this kit has been pre-coated with an anti LRRC15 antibody.

First, the sample is added into the wells of the ELISA plate and allowed to react.

Next after washing, HRP conjugated anti LRRC15 antibody is added to react. After washing, substrate is added to the HRP conjugated anti LRRC15 antibody reacted with LRRC15.

Finally, HRP color development is read with a plate reader to quantify Human LRRC15 in the sample.

【Ⅰ－4】Kit Component

Storage temperature： 2～8℃

	Reagent	Volume	Quantity
1	Anti LRRC15 Antibody Immobilized Plate	96well (8well x 12 strips)	1 plate
2	LRRC15 Standard	200μL	1tube*1
3	Assay Buffer	25mL	1vial
4	Washing Buffer (10X)*2	25mL	1vial
5	HRP Conjugated Anti LRRC15 Antibody (500X)*3	20μL	1tube
6	Substrate Solution	12mL	1vial
7	Stop Solution (2N H_2SO_4)	6mL	1vial
8	Plate Seals		1sheets

*1 Sufficient to create 4 standard curves with n=2.

*2 Crystals may precipitate in the Washing Buffer (10x) during refrigerated storage. Warm the buffer to dissolve it at 45℃ before use.

*3 If the kit is not going to be used immediately, remove the labeled antibody from the kit and store it at -20℃.

Required Materials Not Included in the Kit

・Micropipettes (10 ~ 1000 μL)
・Multichannel micropipette
・Multichannel micropipette Reservoir
・Plate shaker
・Microplate reader (enable to measure at wavelength 450nm)
・Plate washer

【Ⅱ】Preparation of Reagents and Samples

【Ⅱ－1】Preparation of Washing Buffer

・Dilute Washing Buffer (10×) to 10 folds with purified water.

e.g. For 1 plate, add 225 mL of purified water to 25mL of Washing Buffer (10 x) and mix well.

【Ⅱ－2】Preparation of Standard Protein solution

	Concentration (pg/mL)	LRRC15 Standard	Assay Buffer	Dilution factor
A	25000			
B	2500	50μL of A	450μL	10
C	1250	250μL of B	250μL	2
D	625	250μL of C	250μL	2
E	313	250μL of D	250μL	2
F	156	250μL of E	250μL	2
G	78	250μL of F	250μL	2
H	39	250μL of G	250μL	2

・To prepare Solution B, add 450μL of Assay Buffer into 50μL of Human LRRC15 Standard (Solution A), and then mix well (10 times dilution). To prepare Solution C, add 250μL of Assay Buffer into 250μL of Solution B, and then mix well (2 times dilution). Similarly, 2 times dilution series for Solution D through H should be prepared.

・Use 100μL for measurement, using 2 wells for each solution (n=2).

・Diluted Human LRRC15 Standard Solution(39～2500 ng/mL) should be freshly prepared at each time before use.

【Ⅱ－3】Preparation of antibody solution

・Dilute HRP conjugated anti-Human LRRC15 antibody (500x) to 500 folds using Assay Buffer.

e.g. For 1 plate, add 20μL of antibody (500x) into 10mL of Assay Buffer. Mix by inverting the tube.

* Diluted antibody solution should be freshly prepared at each time before use.

【Ⅱ－4】Preparation of Samples

In order to measure LRRC15 protein dissolved in some buffer, the sample can be diluted with assay buffer appropriately. In case that LRRC15 level in serum is analyzed, dilute the serum 4-fold with assay buffer and use as samples.

Samples generating absorbance values greater than that of the highest standard should be further diluted using Assay Buffer and reanalyzed.

【Ⅱ－5】Sample Storage

After sample preparation, store at 2-8°C until measurement.

【Ⅲ】Sample measurement procedure

1. Bring anti LRRC15 antibody solid phased plate and the reagents to the room temperature.
2. Prepare Human LRRC15 Standard solution by serial dilution. (from step【Ⅱ－2】)
3. Add 100μL each of serial diluted LRRC15 Standard solution (39～2500 pg/mL) or Sample solution into the well.
4. Seal the microplate with Plate Seals.
5. Incubate at room temperature for 1 hour on a plate shaker set to 800 rpm.
6. Discard all the reaction solution, and then rinse each well with 300μL of Washing Buffer (from step 【II-1】). Repeat this step for 3 times.
7. Add 100μL each of diluted HRP conjugated anti LRRC15 antibody (from step 【II-3】) to the well.
8. Seal the microplate with Plate Seals.
9. Incubate at room temperature for 1 hour on a plate shaker set to 800 rpm.
10. Discard the reaction solution, and then rinse each well with 300μL of Washing Buffer(from step 【II-1】). Repeat this step for 3 times.

11. Add 100μL of Substrate Solution into each well, and then incubate at room temperature protected from light for 20min for static reaction.
12. Visually confirm the coloring, and then add 50μL each of Stop Solution.
13. Place into the Plate-reader, and read the absorbance of each well on a spectrophotometer at the wavelength of 450nm.
14. Create a standard curve by plotting the absorbance value (y axis) for each LRRC15 Standard concentration against the LRRC15 Standard concentration (x axis).
15. Determine the concentration of the target protein in the sample by interpolating absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of LRRC15 in the sample.

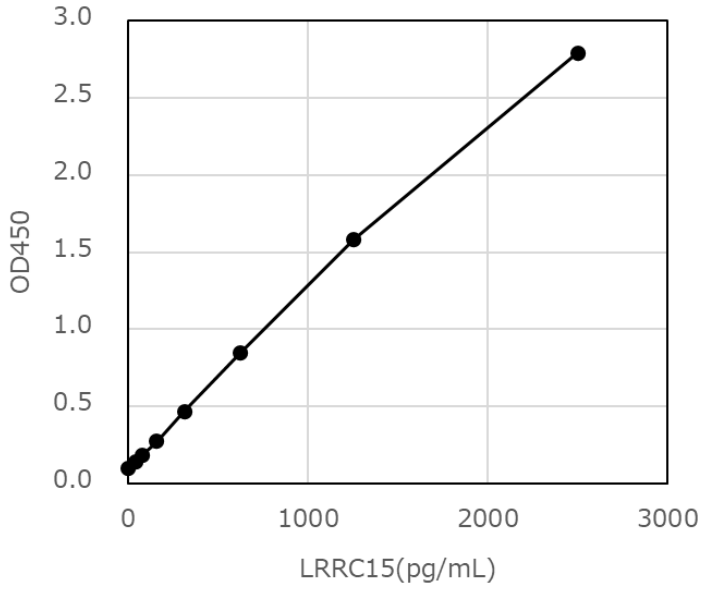
【Ⅳ】Measurement example

【Ⅳ－１】Standard curve

As an example, the graph of absorbance (OD450) against Human Standard LRRC15 concentration is drawn as shown in Figure 1.

However, draw a new standard curve for each assay to calculate the concentration in the sample.

As the Standard LRRC15 is prepared using extracellular domain of LRRC15 fused with some tag sequences, the measured value should be 0.729-fold when convert it as full length LRRC15 protein amount.



LRRC15 (pg/mL)	absorbance(450nm)		mean
	1	2	
0	0.098	0.097	0.098
39	0.139	0.140	0.140
78	0.185	0.188	0.187
156	0.277	0.274	0.276
313	0.469	0.464	0.467
625	0.854	0.842	0.848
1250	1.577	1.585	1.581
2500	2.764	2.816	2.790

Fig.1 Standard curve and measured values

【Ⅳ−2】Sample measurement example

1. Exosomes

Exosomes（31.3, 62.5, 125, 250, 500, 1000, 2000ng/mL）purified from the culture supernatant of Expi293 in which LRRC15 was forcibly expressed were measured.

Figure 2 shows the exosome concentration (ng/mL) and absorbance (OD450) graphically.

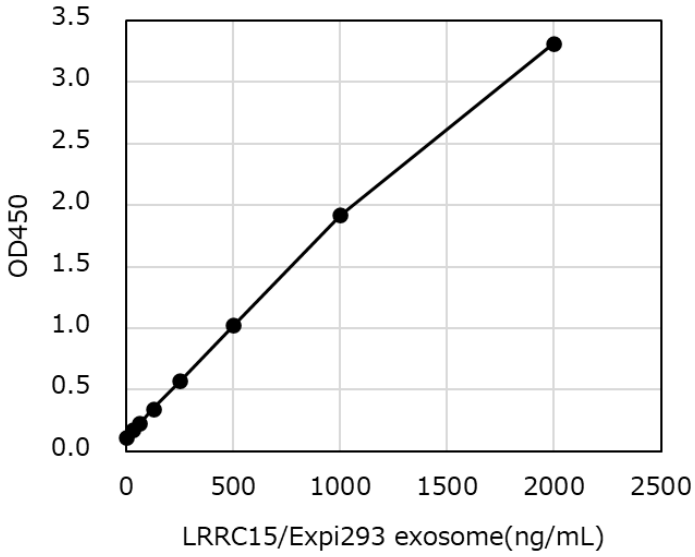


Fig. 2 Measurement of exosomes overexpressing LRRC15

2. Cell extract

The cell extract (1μg/mL) of Expi293 cells overexpressing LRRC15 was prepared with RIPA and measured. Figure 3 shows the culture period and LRRC15 expression level (pg/mL) in the cell extract graphically.

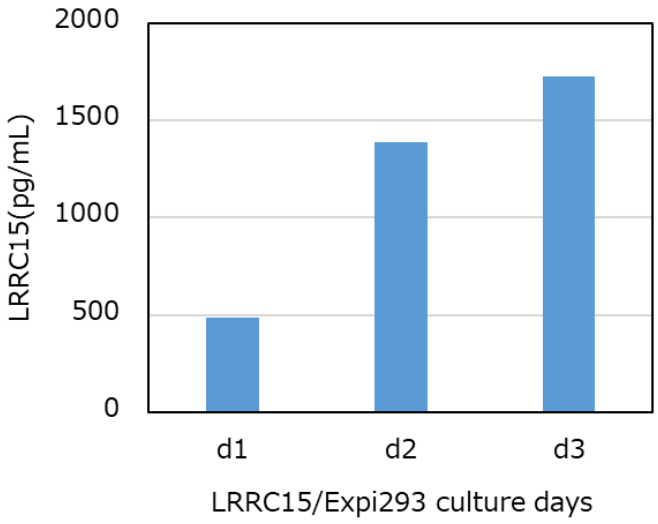


Fig. 3 Time course of LRRC15 expression in the cell extract

【V】Kit expiry date and storage

Expiry date : 6 months after the manufacturing date.

(The manufacturing date is indicated on the kit box label)

Storage : Refrigeration (2-8℃)

【Reference】

1) Purcell JW et al., *Cancer Res.*, **78**, 4059 (2018).