Datasheet for ABIN6720547

LEFTY1 CLIA Kit

4 Images

Go to Product page

Overview

Quantity:	96 tests
Target:	LEFTY1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	13.7 pg/mL - 10000 pg/mL
Minimum Detection Limit:	13.7 pg/mL
Application:	ELISA

Product Details

Purpose:	The chemiluminescent Left-Right Determination Factor 1 / LEFTY1 ELISA kit detects LEFTY1 in human serum, plasma, tissue homogenates and other biological fluids. We offer **validation data** (WB) **for the kit components**. So you can be sure to order a reliable ELISA kit product composed of high quality reagents.
Sample Type:	Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Specificity:	This assay has high sensitivity and excellent specificity for detection of Left/Right Determination Factor 1 (LEFTY1).
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Left/Right Determination Factor 1 (LEFTY1) and analogues was observed.

Product Details

Sensitivity:	5.3 pg/mL
Components:	• Pre-coated, ready to use black 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent • Detection Reagent A • Detection Reagent B • Assay Diluent A • Assay Diluent B • Reagent Diluent (if Detection Reagent is lyophilized) • Substrate A • Substrate B • Wash Buffer (30 x concentrate) • Instruction manual
Material not included:	1. Luminometer capable of reading 96-well microplates with the following parameters: lag time 30.0 secs, read time 1.0 sec/well . 2. Precision single or multi-channel pipettes and pipette tips with disposable tips. 3. Eppendorf Tubes for diluting samples. 4. Deionized or distilled water. 5. Absorbent paper for blotting the microtiter plate. 6. Container for Wash Solution

Target Details

Target:	LEFTY1
Alternative Name:	LEFTY1 (LEFTY1 Products)
UniProt:	O75610

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time. • Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

	• Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. • There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed. • Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. • Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. • Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. • Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.
Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards, 2. Add 100µL standard or sample to each well. Incubate 1 hours at 37 °C,

3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C,
4. Aspirate and wash 3 times,
5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
6. Aspirate and wash 5 times,
7. Add 100µL Substrate Solution. Incubate 10 minutes at 37 °C,
8. Read RLU value immediately.

Reagent Preparation:

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- **Standard:** - Reconstitute the Standard with 0.5 mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 10 ng/mL. Prepare 7 tubes containing 0.25mL Standard Diluent and produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10 ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0 ng/mL.
- **Assay Diluent A and Assay Diluent B** - Dilute 6mL of Assay Diluent A or B Concentrate(2×) with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B. The prepared working dilution cannot be frozen.(In fact, more than 6mL Assay Diluent A and Assay Diluent B are contained in the bottles. Therefore, in every test, please precisely pipette required amount of Diluent and make double dilution in a new container. The prepared working dilution cannot be frozen.)
- **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with working Assay Diluent A or B, respectively (1:100).
- **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).
- **Substrate working Solution** - Mix the substrate A and B by the ratio of 99:1 to make the substrate working solution. Mix thoroughly. For example, prepare 1,000µL Substrate working Solution with 990µL Substrate A + 10µL Substrate B.

Note:

- Making serial dilution in the wells directly is not permitted.
- Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
- Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting.
- The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
- Prepare Substrate working Solution within 15 minutes before assay.
- If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
- Contaminated water or container for reagent preparation will influence the detection result.

Application Details

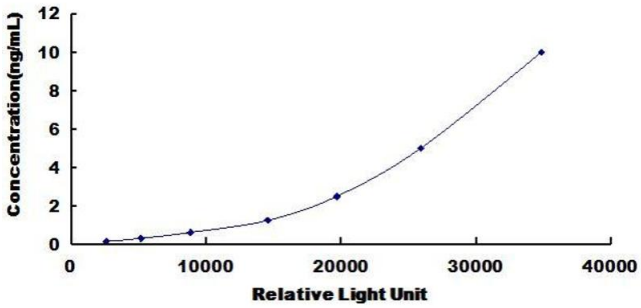
Sample Collection:	**Serum:** Allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 × g. Assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles. **Tissue Homogenates:** The preparation of tissue homogenates will vary depending upon tissue type. For this assay, rinse tissues in ice-cold PBS (0.02 mol/L,pH 7.0-7.2) to remove excess blood thoroughly and weigh before homogenization. Mince the tissues to small pieces and homogenize them in 5-10 mL of PBS with a glass homogenizer on ice (Micro Tissue Grinders work, too). Sonicate the resulting suspension with an ultrasonic cell disrupter or subject it to two freeze-thaw cycles to further break the cell membranes. Centrifugate the homogenates for 5 minutes at 5000 × g. Remove the supernate and assay immediately or aliquot and store at -20°C **Biological Fluids:** Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.
Assay Procedure:	1. Prepare all reagents, samples and standards, 2. Add 100µL standard or sample to each well. Incubate 2 hours at 37 °C, 3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C, 4. Aspirate and wash 3 times, 5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C, 6. Aspirate and wash 5 times, 7. Add 100µL Substrate Solution. Incubate 10 minutes at 37 °C, 8. Read RLU value immediately.
Calculation of Results:	Average the duplicate readings for each standard, control, and samples and subtract the average zero standard relative light unit (RLU). Create a standard curve on log-log graph paper, with LEFTY1 concentration on the y-axis and the RLU value on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, such as curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. In order to make the calculation easier, we plot the RLU value of the standard (X-axis) against

Application Details

	the known concentration of the standard (Y-axis), although concentration is indeed the independent variable while RLU value is the dependent variable. Further, in this part, in order to help the customer perform the assay more visual, we provide the customer with the raw data (not the log of data). However, plotting log of the data to construct the curve will be recommended. The RLU values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). This curve is provided for demonstration only. The customers should establish their own standard curve for each test conducted.
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Left/Right Determination Factor 1 (LEFTY1) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Left/Right Determination Factor 1 (LEFTY1) were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV<10% Inter-Assay: CV<12%
Restrictions:	For Research Use only

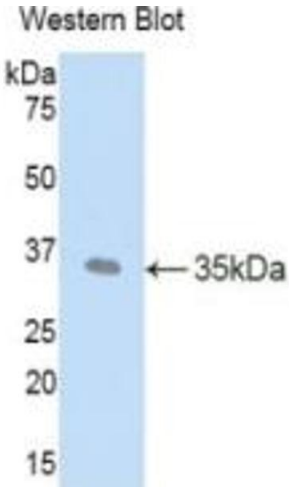
Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C/-20 °C
Expiry Date:	6 months



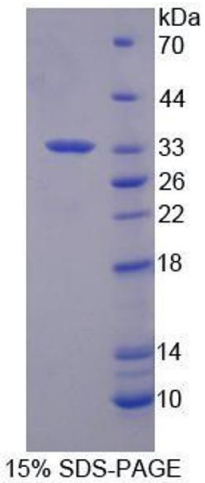
ELISA

Image 1. Typical standard curve



Western Blotting

Image 2. WB of Protein Standard: different control antibodies against Highly purified E. coli-expressed recombinant human LEFTY1.



SDS-PAGE

Image 3. SDS-PAGE of Protein Standard from the Kit (Highly purified E. coli-expressed recombinant human LEFTY1).

Please check the product details page for more images. Overall 4 images are available for ABIN6720547.