Product Manual

Cas13a (CRISPR Associated Protein 13a, C2c2) ELISA Kit

Catalog Number

PRB-5091 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Adaptive immune systems from prokaryotes utilize clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR associated (Cas) proteins to cleave foreign genetic material. Cas13a (formerly C2c2) is part of the Type VI CRISPR-Cas system. When Cas13a is bound to a CRISPR-RNA (crRNA) it forms a crRNA-guided RNA-targeting effector complex. Currently characterized Cas13 proteins contain different enzymatic RNase activities that are required for optimal target activity. When Cas13a binds a precursor-crRNA (pre-crRNA), Cas13a cuts within the crRNA direct repeat in a pre-crRNA array to produce a mature Cas13a-crRNA complex. Subsequently, binding of an RNA molecule (called an activator RNA) complementary to the crRNA causes Cas13a to cut RNA non-specifically through activation of the enzyme's two higher-eukaryotes-and-prokaryotes nucleotide-binding (HEPN) domains to form a single RNase active site (Figure 1).

In addition to its ability to promote bacterial adaptive immunity, the RNA-activated RNA cleavage activity of Cas13a allows for RNA detection and potential diagnostic applications. Catalytically inactive versions of Cas13 may also be useful as programmable RNA binding proteins to regulate or image specific transcripts.

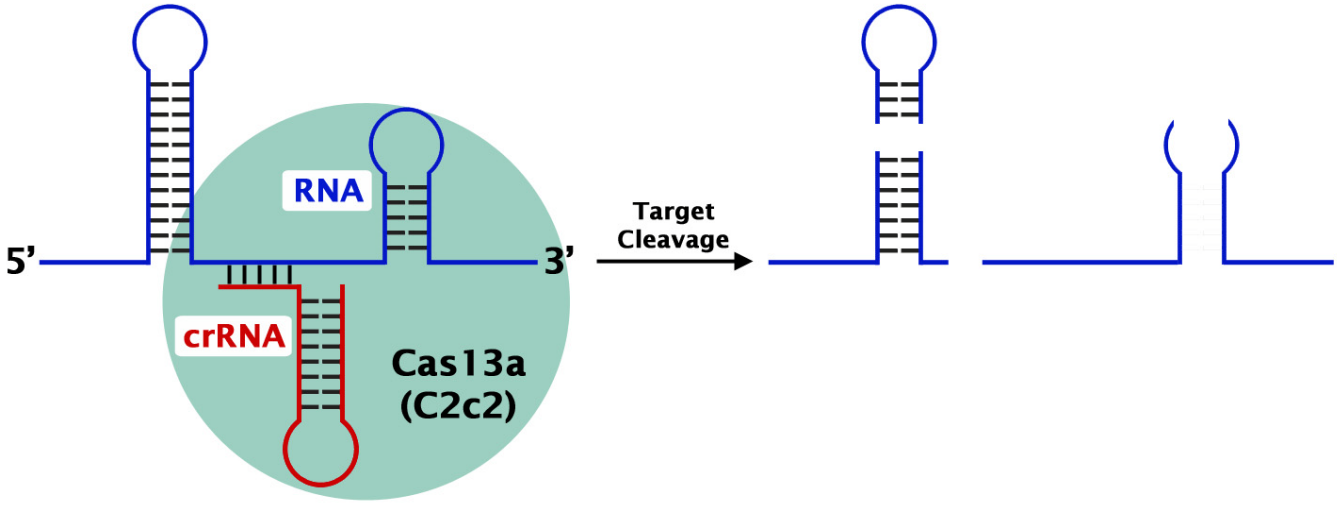


Figure 1: CRISPR/Cas13a (C2c2) RNA Editing.

Cell Biolabs' Cas13a ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of *Leptotrichia shahii* Cas13a in cell or tissue lysate samples. The kit has a detection sensitivity limit of 31 pg/mL Cas13a. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. PRB-5079: Cas9 ELISA Kit
2. AKR-120: GFP Quantitation Kit, Fluorometric
3. AKR-121: GPF ELISA Kit
4. AKR-122: RFP ELISA Kit
5. AKR-130: His-Tag Protein ELISA Kit

Kit Components

1. Anti-Cas13a Antibody Coated Plate (Part No. 50911B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Cas13a Antibody (1000X) (Part No. 50912C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. Cas13a Standard (Part No. 50913B): One 10 µL vial of 1 µg/mL recombinant *L. Shahii* Cas13a.

Materials Not Supplied

1. Cell or tissue lysate
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
7. RIPA buffer

Storage

Upon receipt store the entire kit at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Cas13a Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Cas13a antibody or the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare fresh Cas13a standards before use by diluting in assay diluent. First, dilute the stock Cas13a Standard 1 µg/mL solution 1:10 in Assay Diluent to create a 100ng/mL Cas13a Solution. (e.g. add 5 µL of the stock 1 µg/mL Cas13a Standard to 45 µL of Assay Diluent). Use the 100 ng/mL Cas13a Solution to prepare a series of the remaining Cas13a standards according to Table 1 below.

Standard Tubes	100 ng/mL Cas13a Standard (µL)	Assay Diluent (µL)	Cas13a (pg/mL)
1	10	490	2000
2	250 of Tube #1	250	1000
3	250 of Tube #2	250	500
4	250 of Tube #3	250	250
5	250 of Tube #4	250	125
6	250 of Tube #5	250	62.5
7	250 of Tube #6	250	31.3
8	0	250	0

Table 1. Preparation of *L. Shahii* Cas13a Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Cell or Tissue Lysates: Sonicate or homogenize sample in Lysis Buffer such as RIPA buffer (25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of Cas13a unknown sample or standard to the Anti-Cas13a Antibody Coated Plate. Each Cas13a unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Cas13a antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.

6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Cas13a ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

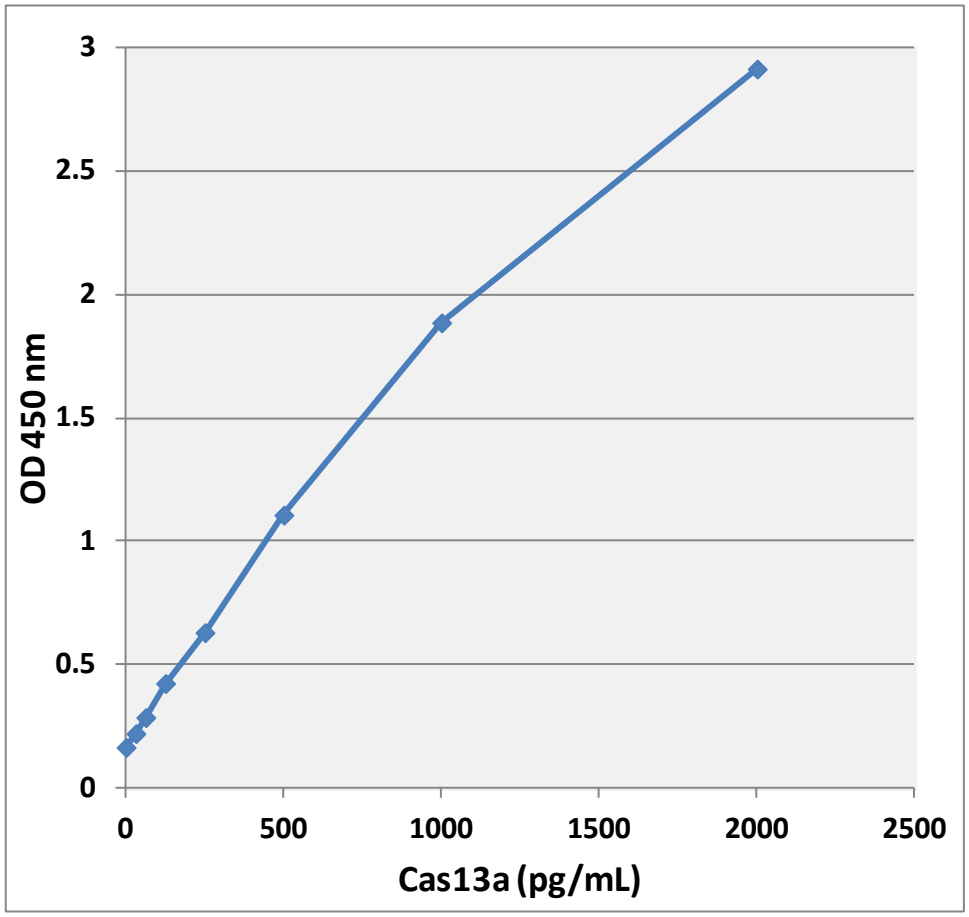
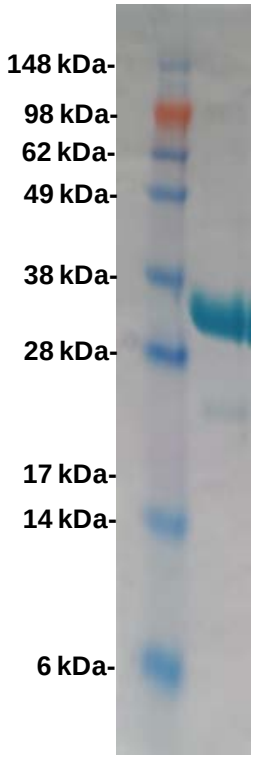


Figure 2: Cas13a ELISA Standard Curve.

A



B

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KNKKMLVEKILNINVDLTVEDIADFVIKELEFWNITKRIEKVKKVNNEFLEKRRNRTYIKSYVLLDKH
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LELESYNSDYIKNLIIELLTKIENTNDTL

Figure 3: Purification of Recombinant Cas13a protein. A. Lane 1: SeeBlue Plus2 MW standard (Invitrogen); Lane 2: Ni-NTA Elution Fraction for Recombinant Cas13a. Purified recombinant

Cas13a protein was used as immunogen to produce the antibodies. **B.** Cas13a Sequence: Recombinant sequence used to make the kit antibody is underlined.

References

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