MANUAL:

Cas 13 Activity Assay Protocol

Catalog Number: orb1742853

Reaction Components:

Active Cas13	It is recommended that the researcher perform a serial dilution of Active Cas13 diluted with 1X Reaction Buffer for optimal results.
Cas13 Reaction Buffer (10x) (Cat #: orb1742843)	Buffer components: 200 mM HEPES pH 7.0, 500 mM KCl, 50 mM MgCl2, 1 mg/mL BSA.
CRISPR RNA	Single guide RNA containing a target-specific spacer sequence and a scaffold sequence responsible for recruiting the Cas enzyme.
Target RNA activator	An RNA oligo that contains the complementary sequence to the crRNA.
Reporter Substrate:	A synthetic RNA oligonucleotide labelled with a fluorophore and a quencher on the two termini.

Assay Protocol:

The RNA trans-cleavage activity of Cas13 is detected in a CRISPR-based fluorescent reporter assay. RNA-guided RNA binding to Cas13 activates the enzyme, inducing target RNA cutting as well as collateral RNase activity. The latter leads to the degradation of the reporter substrate that emits a light signal on cleavage.

Step 1.

Thaw the active Cas13 on ice. Prepare 1X Reaction Buffer. Equilibrate the buffer, guide RNA, target RNA, and reporter substrate to ambient temperature.

Step 2:

Prepare the following working solutions with 1X Reaction Buffer:

- 4X final concentration of Active Cas13

- 4X final concentration of guide RNA
- 2X final concentration of activator/ reporter mix containing the RNA activator and reporter substrate.

Step 3:

In a half-area solid black 96-well plate, add the following components and pre-incubate at room temperature for 10 minutes:

- Component 1. 10 µL of 4X Active Cas13
- Component 2. 10 µL of 4X guide RNA

Note: A blank control can be set up as outlined in step 3 by replacing the enzyme working solution with an equal volume of the assay buffer

Step 4:

To each assay well, add 20 µL of the 2X activator/reporter mix. Shake the plate for 1 minute on a tabletop orbital shaker. Seal the assay wells with microplate sealing tape and incubate at 37°C for 15-30 minutes.

Step 5:

Equilibrate the plate to ambient temperature and then remove the microplate sealing tape. Read fluorescence on a microplate reader.