HBV Ribonuclease H FRET Assay

HBV Biochemical Assays

Authors Information

Cassandra Kukla, Nathan Ponzar, and John Tavis. Saint Louis University, St. Louis, USA
Main author email: cassandra.kukla@health.slu.edu
Senior author email: john.tavis@health.slu.edu

Saint Louis University

Introduction

- This assay detects the activity of HBV RNaseH using fluorescence resonance energy transfer
- The ribonuclease H (RNaseH) substrate is an RNA DNA heteroduplex in which an RNA oligonucleotide contains a fluorophore and the complementary DNA oligonucleotide contains a fluorescence quencher. Catalytic activity of the RNaseH is measured via an increase in fluorescence stemming from release of the fluor upon degradation of the RNA
- Inhibition of RNaseH activity can be determined by comparing the change in fluorescence over time between reactions with and without inhibitors

Materials and Reagents

- HBV Ribonuclease H: This protocol requires purified recombinant HBV RNaseH. Purification of the enzyme is an involved procedure and the protocol is undergoing refinement. Please contact John Tavis (john.tavis@health.slu.edu) for the current protocol and RNaseH expression plasmid.
- Substrate: The substrate for HBV RNase H is a DNA:RNA heteroduplex, prepared as a ratio of 1.1:1 DNA:RNA in 1x RNase H Buffer and annealed for 10 minutes at room temperature following heating the mixture to 90C for 2 min.
    - DNA: 5’-IABkFQ-AGC TCC CAG GCT CAG ATC-3’ (IABkFQ: Iowa Black quencher)
    - RNA: 5’- GAU CUG AGC CUG GGA GCU FAM-3’ (FAM: Fluorescein fluorophore)
- Reagents:
    - -Nuclease free H2O, RNaseOUT (Thermo Fisher)
    - -10x RNase H Buffer (1 M NaCl, 500 mM HEPES pH 8.0)
    - -50 mM MgCl2 (RNase free), DMSO
- Equipment:
    - -Costar 96-well Black Polystyrene Plates (non-treated) (Product # 3694)
    - -Biotek Synergy HTX Multi-mode Reader or equivalent

Experimental Procedures

Assemble the reaction mixture as follows in an RNaseH-free tube, total volume based on number of reactions.

- -Combine per reaction at room temperature: 30.95 uL nuclease free H2O, 9 uL 10x RNaseH Buffer, 0.05 uL RNaseOUT, 10 uL 125 nM substrate, 30 uL HBV RNaseH (the protein amount varies between enzyme preparations, balance enzyme concentration to provide sufficient activity to complete the reaction in 30-45 min).
- -If doing an inhibition experiment: Dilute test compounds (10 uM in 100% DMSO) over the

desired concentration range in 12 uL per dilution.

- -Aliquot 80 uL of reaction mixture into each well of 96 well black polystyrene plate, add 10 uL of diluted compound to each well (or H20 if not doing an inhibition experiment). The plate should include a positive control with no inhibitor and a negative control well lacking enzyme.
- -Add 10 uL 50 mM MgCl2 to each well to initiate the reaction and immediately place the plate into a plate reader that has been pre-heated to 37C.
- -Read the reactions at excitation 485 and emission 528 for 1 hour at 2 minute intervals at 37C. Data analysis: The reaction rate is the slope of the rise in the fluorescence curve during its early linear phase. The first ~3-5 minutes of the reaction may need to be omitted when calculating the slope due to changes in intrinsic fluorescence of fluorescein from the change from room temperature to 37C.

References

This assay has not yet been published.