CREATIVE BIOMART®
Assay Kit

Reverse Transcriptase Assay Kit

Product Information

Cat.No. RTAT-1000

Product Overview

Reverse Transcriptase Assay Kit is a convenient, efficient and inexpensive assay for measuring reverse transcriptase activity. The key to this method is our Green dsDNA quantitation reagent, which preferentially detects dsDNA or RNA-DNA heteroduplexes over single-stranded nucleic acids or free nucleotides. In the assay, reverse transcriptase activity in a biological sample generates long RNA-DNA heteroduplexes from a mixture of a long poly(A) template, an oligo-dT primer and dTTP. The RNA-DNA heteroduplexes formed are then detected by the Green reagent. In less than an hour, samples can be read in a fluorometer or microplate reader with filter sets appropriate for fluorescein (FITC).

Storage

Upon receipt, the kit should be stored at -20°C, protected from light. The 20X TE buffer and the lambda DNA standard are best stored at 4°C; however, either may be frozen for longterm storage. Stored properly, the kit's components should remain stable for at least six months. Allow reagents to warm to room temperature before opening the vials.

Size

1000 microplate assays

Kit Components

Green dsDNA quantitation reagent (Component A), 0.55 mL of 400X dye in DMSO
20X TE buffer (Component B), 12 mL of 200 mM Tris HCl, 20 mM EDTA, pH 7.5
Lambda DNA standard (Component C), 0.55 mL of 100 µg/mL DNA in TE buffer
Poly(A) ribonucleotide template (Component D), 55 µL of 1 mg/mL template in 100 mM TrisHCl, 0.5 mM EDTA, pH 8.1. The template is approximately 350 bases long
Oligo d(T)16 primer (Component E), 55 µL of 50 µg/mL primer in 100 mM TrisHCl, 0.5 mM EDTA, pH 8.1
Polymerization buffer (Component F), 22.5 mL of 60 mM TrisHCl, 60 mM KCl, 8 mM MgCl2, 13 mM DTT, 100 µM
dTTP, pH 8.1

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EDTA (Component G), 2.5 mL of a 200 mM solution in water

Materials Required but Not Supplied

Reverse transcriptase
Enzyme dilution buffer, nuclease-free and appropriate for the selected reverse transcriptase
Nuclease-free water

Assay Protocol

Running the Reverse Transcriptase Reactions
1.1 Anneal the template and the primer. For every 100 reactions, mix together 5 µL of poly(A) ribonucleotide template (Component D) and 5 µL of oligo d(T)16 primer (Component E) in a nuclease-free microfuge tube. Incubate the mixture at room temperature for one hour to allow the primer to anneal to the template.
1.2 Prepare the reaction mixture. Dilute the template/primer solution (prepared in step 1.1) 200-fold into polymerization buffer (Component F). For 100 reactions, dilute 10 µL of template/ primer solution into 2.0 mL of polymerization buffer.
1.3 Aliquot the reaction mixture. For each sample to be assayed, aliquot 20 µL of this reaction mixture into microplate wells or microfuge tubes.
1.4 Prepare the samples of reverse transcriptase. Dilute the samples in an appropriate enzyme dilution buffer, for example, 50 mM Tris-HCl, 20% glycerol, 2 mM DTT, pH 7.6.
1.5 Prepare a standard curve. Prepare a standard curve using a dilution series of a known amount of reverse transcriptase in the enzyme dilution buffer. Note that the fluorescence response of the Green reagent can be effected by the presence of compounds found in biological samples. Thus, to serve as an effective control, the solution used to prepare the standard curve should be treated the same way as the experimental samples and should contain similar levels of such compounds.
1.6 Add the standards and samples to the reaction mixture. Add 5 µL of the dilute enzyme standards or samples (from steps 1.4 and 1.5) to the wells or tubes containing the reaction mixture (aliquoted in step 1.3).
Include a control (no enzyme) sample, by adding 5 µL of enzyme dilution buffer to one reaction sample.
The provided 100 µg/mL lambda DNA solution (Component C) can be used as a reference to

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assess the settings of the instrument or to estimate the amount of RNADNA hybrids produced during the experiment. For the standard, prepare a dilution series of the lambda DNA from 40 ng/mL to 4 µg/mL in enzyme dilution buffer. Substitute 5 µL of each dilution in place of an enzyme sample for final concentrations between 1 ng/mL and 100 ng/mL.
1.7 Run the reaction. Incubate the reaction samples at 25°C for 1060 minutes. To ensure accurate results, all reactions tested simultaneously must be run for the same amount of time.
1.8 Stop the reaction. Add 2 µL of 200 mM EDTA (Component G) to each reaction. Store reaction samples at 4°C until they can be quantitated.

Determining the Reverse Transcriptase Activity
2.1 Prepare 1X TE buffer. Prepare 1X TE buffer by diluting 20X TE buffer (Component B) 20-fold into nuclease-free water. For 100 assays, add 1 mL of 20X TE buffer to 19 mL of nucleasefree water. This buffer is used below for diluting the Green reagent. Because the Green dye is extremely sensitive for the detection of DNA, it is imperative that the 1X TE solution used is free of contaminating nucleic acids.
2.2 Prepare Green working solution. Prepare an aqueous working solution of the Green reagent (Component A) by making a 345-fold dilution of the concentrated DMSO solution into 1X TE (made in 2.1). For 100 samples, add 50 µL of the Green reagent to 17.2 mL of 1X TE.
We recommend preparing this solution in a plastic container rather than glass, as the reagent may adsorb to glass surfaces.
Protect the Green Working Solution from light by covering it with foil or placing it in the dark, as the Green reagent is susceptible to photodegradation.
For best results, this solution should be used within a few hours of its preparation.
2.3 Add Green working solution to the samples. Add 173 µL of Green Working Solution to the reactions from step 1.8.
2.4 Incubate for 2 to 5 minutes at room temperature. Protect the samples from light during the incubation.
2.5 Measure the fluorescence. After incubation, measure the sample fluorescence using a microplate reader and standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all

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samples.

2.6 Calculate the level of reverse transcriptase. Subtract the fluorescence value of the control (no enzyme) sample from that of each of the samples. Use corrected data to generate a standard curve of fluorescence versus reverse transcriptase activity. Determine the reverse transcriptase activity of the experimental samples from the reverse transcriptase standard curve.

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