Revised: 14–March–2002

EnzChek® Reverse Transcriptase Assay Kit (E-22064)

Quick Facts

Storage upon receipt:
- –20°C
- Dessicate
- Protect from light

Ex/Em: 502/523 nm

Number of assays: 1000 microplate assays

Introduction

Molecular Probes' EnzChek® Reverse Transcriptase Assay Kit is a convenient, efficient and inexpensive assay for measuring reverse transcriptase activity. The key to this method is our PicoGreen® 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 PicoGreen reagent. In less than an hour, samples can be read in a fluorometer or microplate reader with filter sets appropriate for fluorescein (FITC). The assay is sensitive, detecting as little as 0.02 units of HIV-1 reverse transcriptase, and has about a 50-fold linear range (Figure 1). Because it is much more rapid and less expensive than standard isotopic assay or immunoassays, it is suitable for testing large numbers of biological samples. Its simplicity also makes it useful for automated high-throughput screening of reverse transcriptase inhibitors.

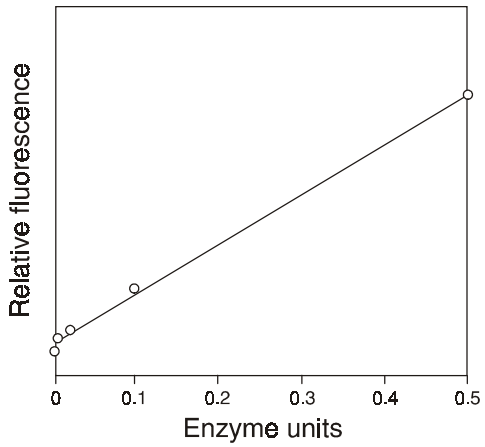

Figure 1. *Detection of HIV reverse transcription using the Reverse Transcriptase Assay Kit, showing detection of 0.02 to 0.5 units of the enzyme. 1 unit = incorporation of 1 nmol of dTTP in 10 minutes at 37 °C, using poly(A) and oligo(dT) as the template and primer, respectively.*

Materials

Contents

- **PicoGreen 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 Tris–HCl, 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 Tris–HCl, 0.5 mM EDTA, pH 8.1
- **Polymerization buffer** (Component F), 22.5 mL of 60 mM Tris–HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 100 µM dTTP, pH 8.1
- **EDTA** (Component G), 2.5 mL of a 200 mM solution in water

Each kit provides sufficient reagent for approximately 1000 microplate assays.

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 long-term 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.

Handling and Disposal

We must caution that no data are available addressing the mutagenicity or toxicity of the PicoGreen dsDNA quantitation reagent. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, solutions of PicoGreen reagent should be poured through activated charcoal before disposal. The charcoal must them be incinerated to destroy the dye.

Materials Required but Not Provided

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

Experimental 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 PicoGreen reagent can be effected by the presence of compounds found in biological samples (Table 1). 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 assess the settings of the instrument or to estimate the amount of RNA–DNA 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 10–60 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.

Table 1. *Effects of several compounds on the signal intensity of the PicoGreen reagent.*

Compound	Concentration	% Signal Change*
Salts		
Ammonium acetate	50 mM	3% decrease
Sodium acetate	30 mM	3% increase
Sodium chloride	200 mM	30% decrease
Zinc chloride	5 mM	8% decrease
Magnesium chloride	50 mM	33% decrease
Urea	2 M	9% increase
Organic Solvents		
Phenol	0.1%	13% increase
Ethanol	10%	12% increase
Chloroform	2%	14% increase
Detergents		
Sodium dodecyl sulfate	0.01%	1% decrease
Triton X-100	0.1%	7% increase
Proteins		
Bovine serum albumin	2%	16% decrease
IgG	0.1%	19% increase
Other Compounds		
Polyethylene glycol	2%	8% increase
Agarose	0.1%	4% increase

* The compounds were incubated at the indicated concentrations with PicoGreen reagent in the presence of 500 ng/mL calf thymus DNA. All samples were assayed in a final volume of 200 µLin 96-well microplates using a CytoFluor® microplate reader. Samples were excited at 485 nm and fluorescence intensity was measured at 520nm.

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 nuclease-free water.

- This buffer is used below for diluting the PicoGreen reagent. Because the PicoGreen 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 PicoGreen working solution. Prepare an aqueous working solution of the PicoGreen 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 PicoGreen 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 PicoGreen Working Solution from light by covering it with foil or placing it in the dark, as the PicoGreen reagent is susceptible to photodegradation.

- *For best results, this solution should be used within a few hours of its preparation.*

2.3 Add PicoGreen working solution to the samples. Add 173 µL of PicoGreen 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 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.

References

1. Anal Biochem 246, 228 (1997).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
E-22064	EnzChek® Reverse Transcriptase Assay Kit *1000 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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