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A Geno Technology, Inc. (USA) brand name

RNase-DETECT™

A Detection Assay for RNAse Activity

(Cat. # 786-115, 786-116)

INTRODUCTION

The RNase-DETECT™ kit detects RNase activity in any reagent solution that has the potential for RNase contamination. It is a straightforward, non-radioactive-based assay that is simple to perform yet offers sensitivity down to the femtogram level of RNase. You control the level of sensitivity by the period of time you allow for the reaction to incubate. Allowing overnight incubations at 40°C will result in maximal sensitivity (into the femtogram range). Everything is provided in the kit for simple and efficient detection of RNase activity in your solution of concern.

ITEM(S) SUPPLIED

Description	**Cat. # 786-115 50 Tests**	**Cat. # 786-116 100 Tests**
RNA Substrate Tubes	7 x 8 tube strips	14 x 8 tube strips
RNase Free Tubes	7 x 8 tube strips	14 x 8 tube strips
Molecular Grade Water	1.8ml	2 x 1.8ml
2X Reaction Stop Dye	1.8ml	2 x 1.8ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit at -20°C. When stored and used properly, it is stable for 1 year.

PREPARATION

1. Prepare an RNase-free bench area by washing the work surface thoroughly with an RNase removal agent, e.g. RNase OUT (Cat # 786-70) or a similar product.
2. Make sure that pipetting devices and microfuge tube racks to be used are RNase-free by cleaning thoroughly - see step 1.
3. Set an incubator at 40°C for use in the test reaction(s).
4. RNA Substrate Tubes and RNase Free Tubes can be used as 8 tube strips or, if necessary, single tubes can be cut from the strips.

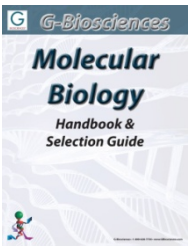
PROTOCOL

1. Spin the RNA Substrate tube to be used for 10 seconds in a microfuge to pellet any loose substrate. Add 20µl of Molecular Grade Water (DNase- and RNase-Free) to the tube and, label this as Tube A. Vortex mix, spin briefly in a microfuge, and let stand at room temperature for 2 minutes to allow the RNA to hydrate. Then place the tube on ice.
2. Transfer 10µl of the contents of Tube A to one of the RNase-free tubes provided in this kit and label it as Tube B. This will be the test reaction tube while Tube A will be your control.
3. Add 5µl of the solution to be tested to Tube B. To control Tube A add 5µl of Molecular Grade Water (RNase-free).

4. Incubate the tubes for 2 hr at 40°C (or longer depending on how low a level of RNase activity you wish to detect. See step #8).
5. Add 15µl of 2X Reaction Stop Dye to both Tube A and B. Mix and spin briefly in microfuge.
6. Load 8µl of the samples on a 2% agarose gel and electrophorese for 5 minutes at 100 volts constant voltage.
7. After 5 minutes, stop the current and load 4µl of each sample in the same lanes and repeat the 5 minute run as above.
8. After this 5 minute run repeat step 7 with a 2µl sample and examine the gel after 5 minutes. These short electrophoresis times are sufficient to see sharp bands of RNA at their peak intensity before diffusion starts to weaken the visible signal.
   NOTE: *As bands begin to migrate further and diffuse, it amplifies any RNA degradation.*
   WARNING: *Remember to turn off the current to the gel box prior to gel loading.*
9. Check the gel lanes on a UV transilluminator to determine if the test sample B shows a 50% or greater decrease in ethidium bromide fluorescence relative to the control sample A which only received addition of nuclease-free water.
10. If the fluorescence levels are equivalent in A and B, there is no significant RNase contamination (ie. below 1ng/ul). To achieve enhanced sensitivity of detection, incubate the reaction tubes 4 to 18 hours at 40°C (in our hands we detect pancreatic RNase at a level of 10fg/ul after overnight incubation). If a lower fluorescence in B vs control A is observed, the sample solution tested is contaminated with RNase activity and appropriate action should be taken to remedy the situation. (Preparation of solutions with DEPC-treated deionized water, followed by autoclaving the solution, when possible, is an effective way to maintain an RNase-free state. Those solutions that are sensitive to DEPC and/or autoclaving may be filtered through a 0.22-micron nitrocellulose filter membrane to adsorb contaminating RNases. Following filtration, the solution may then be tested with RNase-Detect™ to insure that your solution is RNase-free).

RELATED PRODUCTS

Download our Molecular Biology Handbook.



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