RNase Contamination Assay Kit

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E3320S 004121113111

E3320S

50 reactions **Lot: 0041211**

Store at –20°C **Exp: 11/13**

Description: Evaluation of RNase contamination is necessary for reagents to be used in experiments with RNA. The RNase Contamination Assay Kit detects general RNase activities including non-enzyme based RNA degradation due to heavy metal contamination in samples and high pH. The assay probe is a fluorescein labeled RNA transcript (300-mer). After incubation with a reagent sample the integrity of the RNA probe is analyzed on denaturing PAGE followed by SYBR Gold staining or preferably by scanning with a FAM/Fluorescein capable imaging system like the Typhoon scanner. Most samples (e.g. enzymes, column fractions from enzyme purifications, unlabeled RNA/DNA samples, buffers, or diluents) can be tested directly. The assay is semi-quantitative and is more sensitive if detergent is present.

In addition to RNase assay, the labeled RNA probe can also be used in studies involving Poly(A) and Poly(U) tailing, RNA ligation, RNA capping/decapping assays and sequence and structure specific RNase assays.

Advantages:

- Convenient gel-based assay
- RNase contamination is clearly visualized
- Can be used with any reagent not interfering with fluorescein imaging
- Can be loaded directly on gel

Applications:

- Evaluation of RNase contamination

Kit Components:

Fluorescein-RNA Probe	50 µl (40 ng/µl)
10X NEBuffer 4	1.5 ml
RNA Loading Dye (2X)	1 ml
Proteinase K	50 µl (4 mg/ml)

Materials not included:

Nuclease-free water, incubator, PAGE/Urea gel, TBE running buffer, power supply, SYBR Gold, Typhoon scanner and UV light box.

Fluorescein Labeled RNA Probe:

The probe is an *in vitro* RNA transcript (300-mer), internally labeled with Fluorescein-12-UTP, supplied at 40 ng/µl. The sequence of the transcript can be found on the product page at www.neb.com.

1X NEBuffer 4:

50 mM Potassium acetate
20 mM Tris-acetate
10 mM Magnesium acetate
1 mM Dithiothreitol
(pH 7.9 at 25°C)

2X RNA Loading Dye:

95% Formamide
0.02% SDS
0.02% Bromophenol blue
0.01% Xylene cyanol
1 mM EDTA

Proteinase K:

4 mg/ml

Protocol:

1. Preparation of test samples

Each 10 µl standard reaction uses 1 µl of test sample. The test sample should be diluted in sample storage buffer if it is very concentrated (e.g. enzyme stock). It is recommended that a reaction with a sample diluent (e.g. enzyme storage buffer) as well as a water control should be included along with test samples. The recommended minimal reactions are three.

If a custom reaction buffer is used for sample testing, replace 10X NEBuffer 4 with 10X custom reaction buffer. Be sure to include appropriate controls. If the test sample is water, we recommend replacing the water volume with sample water (8 µl). Be sure to include a control reaction with RNase-free water. Samples containing very high concentration of salt or organics may inhibit RNase activity and interfere with PAGE.

2. Assay set up

It is highly recommended that a master mix with enough overage is prepared. Table 1 shows an example of a master mix preparation. After combining the components, mix them thoroughly by vortexing or by flicking the tube several times and then pulse-spin briefly. Aliquot 9 µl of master mix to each tube and then add 1 µl of appropriate controls and test samples to the respective tubes. Mix well.

Table 1. Reaction Set Up for RNase Contamination Assay

Components	Each Rxn	10 Reactions with 10% Overage
Water	7 µl	77 µl
10X NEBuffer 4	1 µl	11 µl
RNA probe	1 µl	11 µl
Total volume	9 µl	99 µl

3. Incubate the reactions in a dry air incubator at 37°C for 1 – 16 hours (overnight)

Incubation time varies depending on the quality requirements of test samples. More specifically, it depends on the applications of the test sample. For example, if the NTPs will be used in *in vitro* transcription at 37°C for 16 hours, the NTPs should be incubated for 16 hours. Because the assay is more sensitive when detergent is present, 2 hour incubation is sufficient for samples containing detergent.

4. Proteinase K treatment to release bound RNA probe (optional)

If the test sample can potentially bind to the RNA probe, e.g. MMLV reverse transcriptase, the test reaction should be treated with 1 µl of proteinase K at room temperature for 5–10 minutes before gel analysis. This treatment will release the bound RNA probe and help with gel image analysis. You may choose to treat all of the reactions with proteinase K when you are unsure whether the test samples will bind to the RNA probe. Proteinase K treatment does not interfere with the assay. Figure 1A shows the advantage of PK treatment of reactions with samples that bind to the RNA probe (reactions 1 and 2) and no difference for reactions with samples that do not bind to RNA probe (control and reaction 3).

5. Stop reaction by adding 10 µl of 2X RNA loading dye to each reaction

Add 10 µl of 2X RNA loading dye to each reaction. Mix thoroughly and pulse-spin briefly. If you are unable to proceed with gel analysis, store the samples at –20°C. Samples can be stored at –20°C for at least three days.

6. Gel electrophoresis

We recommend analyzing the reactions on a denaturing polyacrylamide gel (PAGE-Urea gel, 5–10%). Before loading, heat the samples at 65–70°C for 5–10 min, pulse-spin briefly. Load 10 µl of each sample onto gel. Run the gel until the bromophenol blue (the fast dye) is approximately 2–3 cm from the bottom of the gel. If the gel runs too far, the free label will run off the gel. For a standard mini-gel, the running time is about 30–45 min.

7. Visualization and analysis

a) <u>On a fluorescein capable imaging system</u>

The integrity of the fluorescein labeled RNA probe can be visualized by a fluorescein capable imaging system directly. After electrophoresis, remove the gel carefully and scan it on the Typhoon scanner or equivalent. Compare the RNA probe intensities of test samples to that of a valid water control. If the RNA probe is mostly intact, the sample passes the RNase contamination assay (e.g. reactions 2 and 3 in Figure 1A).

1A.

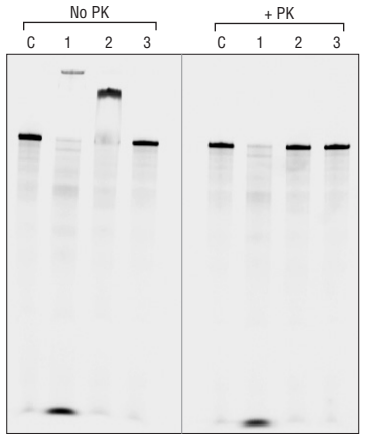

Figure 1A: *Proteinase K treatment releases bound RNA probe. After incubation for 16 hours at 37°C, control and test reactions were analyzed on 6% PAGE-Urea gel. The gel was imaged on a Typhoon scanner. PK treatment releases bound RNA probe (reactions 1 and 2). The +PK panel shows reactions 2 and 3 with intact RNA probe, indicating that the samples are clean. Reaction 1 shows heavy degradation with free label at the bottom, indicating that the sample is contaminated with RNase activity.*

(see other side)

CERTIFICATE OF ANALYSIS

b) By SYBR Gold staining

If a Typhoon scanner is not available the gel can be stained with SYBR Gold and visualized on a UV light box. Carefully remove the gel and stain it in 1X SYBR Gold for 5–10 min with occasional shaking. Rinse the gel briefly with water and visualize it on a UV light box. Figure 1B shows the image of the same gel in Figure 1A stained by SYBR Gold. Note in Figure 1B, the free label in reaction 1 is barely visible. Since SYBR Gold stains nucleic acids, samples containing DNA or RNA will interfere with the visualization.

1B.

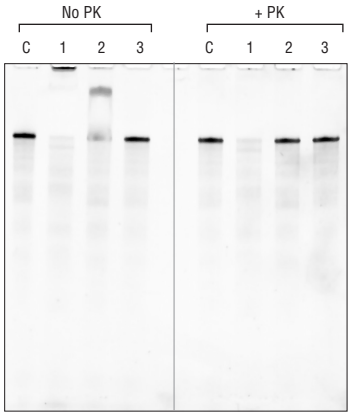

Figure 1B: *The same gel in Figure 1A stained with SYBR Gold. Note the free label in reaction 1 is less visible.*

Companion Products Sold Separately:

Proteinase K
#P8102S 60 mg

RNA Loading Dye (2X)
#B0363S 4 x 1 ml