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Product Information

Ribonuclease A Detection Kit

Catalog Number **RN0100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The Ribonuclease A Detection Kit provides ready-to-use reagents for detecting the presence of Ribonuclease A activity.

Ribonuclease A converts RNA to oligonucleotides in the presence of water. During incubation of the Ribonuclease A sample and RNA at 25 °C, the reaction is followed by monitoring the decrease in absorbance at 300 nm.

The assay can be performed using cuvettes or plates. The procedure provided is for detection using cuvettes with a 1 mL reaction volume.

Components
Each kit contains sufficient reagents for 100 one mL assays.

Reaction Buffer (Catalog Number R4783) 100 mM Sodium Acetate, pH 5.0, at 25 °C	2 × 500 mL
RNA (Catalog Number R6750)	500 g
Ribonuclease A Control (Catalog Number R5500)	2 × 10 g

Reagents and Equipment Required but Not Provided.
- Pipettes and tips
- Ultrapure water
- Cuvettes or 96 well plates
- Containers for dilution
- Appropriate instrument to measure absorbance at 300 nm at constant temperature of 25 °C.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
The Reaction Buffer is provided as a ready-to-use solution.

0.1% (w/v) RNA Solution – Prepare a 1 mg/mL solution of RNA in Reaction Buffer using the RNA provided in the kit (Catalog Number R6750). Ensure dissolution by swirling or inversion. Do not use a stir bar. Dissolution may take up to 30 minutes. Upon dissolution, verify the concentration of the substrate (see Procedure).

Ribonuclease A Solution for Total Hydrolysis determination (enzyme control) – Immediately before use, prepare a solution containing 0.5–0.75 Kunitz units/mL of Ribonuclease A (Catalog Number R5500) in cold ultrapure water. Consult the CofA for activity of Ribonuclease A control.

Ribonuclease A Solution (enzyme control) – Immediately before use, prepare a solution containing 0.2–0.3 Kunitz units/mL of Ribonuclease A (Catalog Number R5500) in cold ultrapure water. Consult the CofA for activity of Ribonuclease A control.

Test Samples – Immediately before use, prepare a solution containing 0.2–0.3 Kunitz units/mL of sample in cold ultrapure water.

Storage/Stability
The Reaction Buffer is stable for at least 2 years at 2–8 °C.

The RNA (Catalog Number R6750) should be stored at 2–8 °C.
The Ribonuclease A (Catalog Number R5500) should be stored at 2–8 °C and is stable for at least 6 months.

For long term storage the RNA (Catalog Number R6750) and Ribonuclease A control (Catalog Number R5500) should be stored at –20 °C.

Procedure
The researcher must determine the optimal procedure conditions for the Ribonuclease A specific to their application.

1. Verify the concentration of the RNA by preparing a cuvette with 500 µL of the RNA solution and 500 µL of ultrapure water. Mix by inversion.
2. Prepare a blank cuvette by preparing a cuvette with 500 µL of Reaction Buffer and 500 µL of ultrapure water.
3. Read the absorbance at A_{300nm} of the RNA cuvette vs. the blank cuvette.
4. The absorbance must be 0.73±0.025 prior to beginning the assay. Adjust with the appropriate amount of Reaction Buffer or RNA.
5. Determine total hydrolysis (E_f)
   a. Pipette 0.5 mL of RNA Solution and 0.5 mL of Ribonuclease A Solution (0.5–0.75 Kunitz units/mL) into three cuvettes (for triplicate readings).
   b. Blank the spectrophotometer against the cuvette prepared in step 2.
   c. At 25 °C, read the absorbance of the triplicate cuvettes for ~120 minutes at 1 minute intervals or until the ΔA_{300nm}/min is <0.002.
6. Rate determination (E_0)
   a. Blank the spectrophotometer against the blank cuvette prepared in step 2.
   b. Pipette 500 µL of RNA solution into a cuvette. Add 460 µL of ultrapure water to the cuvette.
   c. Mix by inversion and equilibrate to 25 °C. Monitor the A_{300nm} until constant.
   d. Add 40 µL of Ribonuclease A Solution (enzyme control) (0.2–0.3 Kunitz/mL in ultrapure water).
   e. Immediately mix by inversion and record the decrease in A_{300nm} for ~10 minutes. Determine the slope of the line (ΔA_{300nm}/min).
7. Repeat rate determination (E_0) with unknown samples, replacing the Ribonuclease A solution with the unknown solution.

Plot $\ln(E_0 - E_f)$ versus time (minutes) and determine the slope of the line

$$\text{Slope} = \Delta\ln(E_0 - E_f)/\Delta t$$

Calculate the Kunitz units/mL enzyme:

$$\frac{(\text{slope})\ (\text{df})\ (V_F)}{(V_E)}$$

df = Dilution Factor
V_F = Total Volume of assay (in milliliters)
V_E = Volume (in milliliters) of enzyme used

Reference
1. Kunitz, M., J. Biol. Chem., **164**, 568 (1946).

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