PRODUCT: FORMAZOL®

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PRODUCT DESCRIPTION

FORMAZOL® is a specially purified and stabilized formamide for use as an RNA solubilizer. Unlike any other commercially available formamide, FORMAZOL is stable and can be used without additional purification for RNA solubilization. It remains stable for two years when stored at 4 C or -20 C.

Stabilized formamide has several advantages over water as an RNA solubilization agent. (1) RNA solubilized in FORMAZOL is protected from degradation by RNase. (2) It can be stored for at least one week at room temperature or five years at -20 C instead of -70 C as required for RNA solubilized in water. (3) A high sample volume (up to 50% of slot volume) can be applied to a formaldehyde-agarose gel for electrophoretic separation or RNA samples [P. Chomczynski (1992), Nucleic Acid Res 20, 3791-3792].

APPLICATION NOTES

<u>RNA solubilization</u>. Drying of RNA to evaporate residual amounts of ethanol is not recommended. Add 100 μ l of FORMAZOL to a 100 - 400 μ g RNA sample. Solubilize RNA by repetitive pipetting and incubation at 50 - 55 C for 10 - 15 minutes.

<u>RNA quantitation</u>. FORMAZOL contributes to the optical density readings of RNA. To normalize for this effect, mix an aliquot of the RNA sample (3 - 10 μ l) with 1 ml water or 1 - 3 mM Na₂HPO₄. Prepare a blank sample with an equal aliquot of FORMAZOL and diluent. Measure the A₂₆₀ of the RNA sample against the blank sample.

RNA dissolved in FORMAZOL has a slightly different UV spectrum than RNA dissolved in water. In water, 1 OD260 = $40 \ \mu g \ RNA$; in FORMAZOL 1 OD260 = $36 \ \mu g \ RNA$. The 260/280 ratio of RNA in FORMAZOL is 11% lower than in water.

<u>Formaldehyde-gel electrophoresis.</u> Prepare a formaldehyde reaction solution containing: water (87 μ l), formaldehyde (81 μ l), bromophenol blue (0.25 mg/ml) in 50% glycerol (48 μ l), and 20X MOPS buffer (24 μ l). The formaldehyde reaction solution is highly unstable and should be prepared immediately before use. Mix equal volumes of the RNA sample solubilized in FORMAZOL with the formaldehyde reaction solution. Incubate at 55 C for 15 minutes. Apply this solution on a formaldehyde-agarose gel and perform the electrophoresis according to your standard protocol.

<u>RT-PCR</u>. RNA solubilized in FORMAZOL can be used for RT-PCR by adding it directly to the reverse transcription reaction mix or after precipitation of RNA from FORMAZOL. When RNA in FORMAZOL is added directly to the reaction, the final concentration of FORMAZOL in the reverse transcription reaction mix should not exceed 5% of the total reaction volume. At higher concentrations, FORMAZOL may inhibit reverse transcriptase.

<u>RNA precipitation</u>. When necessary, precipitate RNA from FORMAZOL by adding NaCl (do not use sodium acetate) to a final concentration of 0.2 M followed by 4 volumes of ethanol. Store samples 3 - 5 minutes at room temperature and centrifuge at 10,000 g for 5 minutes. Remove supernatant and dissolve RNA pellet in water.

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