BIOCHAIN

39600 Eureka Dr.
Newark, CA 94560 USA
Email:

Tel: 1-888-762-2568
Fax: 510-783-5386
Web: www.biochain.com

Placenta Ribonuclease Inhibitor

Catalog No.: **Z5040001**
Lot No.:
Sorce: Human Placenta
Size: 5,000 units
Concentration: 40 u/µl
Storage Buffer: 20 mM HEPES-KOH (pH 7.5), 50 mM KCl, 5 mM DTT and 50% glycerol.
Unit Definition: One unit is the amount of enzyme required to inhibit by 50% the activity of 5 ng of RNase A at 25℃ (This inhibitor activity is determined by its ability to inhibit hydrolysis of cyclic 2', 3'-CMP by RNase A)
Quality Control: Nuclease activity was not detected in any of the following cases, as judged from the intact gel electrophoresis:

1. After incubation of 1 µg of λ DNA-Hind III fragments with 300 units of enzyme for 24 hours at 37℃
2. After incubation of 1 µg of pBR322 DNA with 300 units of enzyme for 24 hours at 37℃
3. Migrates as a single band of 50 kd in SDS-polyacrylamide gel electrophoresis.

Note: Enzyme inhibits in a wide pH range, but most strongly at pH 7-8. Requires DTT of at least 1 mM to be active.

Storage and Handling: -20℃
Shelf Life: One year from the date of purchase if stored and handled properly

Protocol

A. Transcription in vitro (unlabeled RNA)

The standard in vitro transcription assay below uses Ribonuclease Inhibitor at a final concentration of 1 u/µl. With appropriate modifications, this reaction can be used for in vitro transcription analysis in a variety of experimental applications.

5X transcription buffer	20 µl
DTT, 100mM	10 µl
Ribonuclease Inhibitor	100 u
ATP, GTP, CTP and UTP, 2.5 mM each	20 µl
linearized plasmid DNA, 2–5 µg in H_2O or TE buffer	2 µl
RNA Polymerase; SP6, T3 or T7	50 u
Nuclease-free water	X µl
Final volume	100 µl

Incubate for 60–120 minutes at 37-40°C.

B. Transcription in vitro(^{32}P-labeled RNA probes)

5X transcription buffer	4 µl
DTT, 100mM	2 µl
Ribonuclease Inhibitor	20 u
ATP, GTP and UTP, 2.5 mM each	4 µl
CTP, 100 µM	2.4 µl
Linearized template DNA, 0.2–1.0mg/ml in H_2O or TE buffer	1 µl
[α^{32}P]CTP, 50µCi at 10mCi/ml	5 µl
RNA Polymerase, SP6, T3 or T7	1 µl
Nuclease-free water	X µl
Final volume	20 µl

Incubate for 60 minutes at 37–40°C.

III. Composition of Buffers and Solutions

5X transcription buffer
200 mM Tris-HCl (pH 7.5)
30 mM $MgCl_2$
10 mM spermidine
50 mM NaCl
1X TE buffer
10 mM Tris-HCl (pH 8.0)

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1 mM EDTA