

siRNA-intact™ Manual

PRODUCT SUMMARY

Cat. No: SI35100

Size: 100 units in 100 ul

Description: siRNA-intact provides optimal protection of siRNA from degradation due to contamination by ribonucleases.

Shipping and Storage: siRNA-intact is shipped frozen. For maximum stability and long-term use, store at -20°C upon receipt. siRNA-intact is stable for six months when stored properly.

Unit Definition: one unit is the amount of siRNA-intact required to inhibit by 50% the activity of 5 ng of ribonuclease A at 25°C. *Assay Conditions:* 0.1M Tris-acetate, pH 6.5, 1mM EDTA, 1.0 mM cyclic 2',3'-cytidine monophosphate. *Reaction volume:* 1.0 ml. Inhibitor activity is determined by the inhibition of RNase A hydrolysis of cyclic 2',3'-cytidine monophosphate in a spectrophotometric recording assay.

Storage Buffer: 20 mM HEPES-KOH, pH 7.6, 50 mM KCl, 5mM dithiothreitol and 50% glycerol.

Molecular Weight: ~50,000 by SDS-PAGE.

Optimal pH: 5-9 (highest activity at pH 7-8).

METHODS AND PROCEDURES

1. Use 1 unit for 250 ul of transfection volume. Please refer to Table 1 for example transfection reactions.
2. Denaturing conditions (*i.e.* urea, temperatures > 50°C) should be avoided as they may cause release of active ribonuclease from the complex.
3. Ribonuclease inhibitor can be removed by phenol extraction or inactivated by heating at 65°C for 10 minutes.

Tissue culture plate size	Total transfection volume / well	Amount of siRNA-intact / well
96 wells	100 µl	0.5 µl
48 wells	200 µl	1 µl
24 wells	500 µl	2 µl
6 wells	1000 µl	4 µl

Table 1: Suggested siRNA-intact Volumes for siRNA Transfection

References:

Blackburn, P. (1979) Ribonuclease inhibitor from human placenta: rapid purification and assay *J.Biol.Chem.* **254** (24): 12484-12487.

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