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GeneDetectTM One-Step ANTISENSE RNA probe synthesis template (*OptiScript*TM T7 promoter)

Code: GD1001-RT (produces 80mer antisense riboprobe®)

Target: Your target gene mRNA

Probe specificity: Antisense riboprobe® transcribed from the template

hybridizes to nucleotides xxx-xxx located within the

coding region of NM XXXXX.

Hybridization parameters: Tm, XXX, internal stability 3' delta G, -XXX

GC clamp, XXX

Template supplied as: 100mer dsDNA containing *OptiScript*TM T7 promoter

Form: Lyophilized Size: 50ug per vial

Purification: Gel purified, >99% double stranded, full length

Quality Control: By gel electrophoresis

Handling/Storage: Reconstitute in 150ul autoclaved water or TE buffer.

Store at -20°C (DO NOT use DEPC-treated solutions).

Applications: In vitro transcription of antisense riboprobe[®].

Effective Concentration: Use 0.5-1.0ug of template in a standard 10ul

Riboprobe[®] In vitro transcription reaction with 40U of

either T7, T3 or SP6.

Suggested Use: Perform the in vitro transcription reaction at 30°C. This

will increase the percentage of full length riboprobe® transcribed from the template. To reduce premature termination of transcription always ensure limiting

dNTP concentration > 12uM.

Perform in vitro transcription with high quality

reagents.

Promega Riboprobe[®] System – T7, Cat # 1440 Promega Riboprobe[®] System – T3, Cat # 1430 Promega Riboprobe[®] System – SP6, Cat # 1420

Storage and stability.

Standard lyophilized GeneDetectTM One-Step RNA probe synthesis templates are stable for many years if they are stored frozen at -20°C.

A slightly alkaline buffer prevents possible depurination of the template. In an acid buffer A and G bases are eliminated, leading to template damage. TE buffer is the preferred solution for template storage. However nuclease free distilled water can be used in place of TE buffer.

While templates are very stable they are prone to BACTERIAL attack. Use <u>autoclaved</u> tips and tubes.

Storage versus (stability)

Dissolved at 25°C (1 week to 3 months)
Lyophilized at 25°C (2 months to 1 year)
Dissolved (<<15μM) at –20°C (1 year to 3 years)
Dissolved (>15μM) at –20°C (3 years+)
Lyophilized at –20°C (indefinite)

Repeated freeze-thawing can dramatically lower the shelf life of the template.

Resuspending the GeneDetect[™] One-Step RNA probe synthesis template

Centrifuge the tube for a few seconds to ensure the template is fully collected at the bottom of the tube. Carefully open tube, add an appropriate amount of <u>TE buffer or sterile nuclease free water</u> (150ul) close, allow to rehydrate for 2 minutes. Mix the tube by hand for a minute before using a vortex on the solution for 15 seconds. Re-centrifuge the solution. Aliquot and/or store the template.

All scientific enquiries to Scientific@GeneDetect.com