2x AuroraNovo Realtime PCR mix R

Product information:
2x AuroraNovo Realtime PCR mix R is a Taq DNA polymerase-based real-time PCR mix, which contains all components, except for the primer. This reagent is applicable for intercalation assay with SYBR® Green I. This reagent can be used in glass capillary systems (e.g., LightCycler™, Roche Molecular Systems, Inc.). This reagent can be used in a passive reference system (e.g., ABI PRISM® 7700, Applied Biosystems, Inc.). The passive reference dye does not affect any other systems. Hot Start technology with anti-Taq DNA polymerase antibodies enables high specificity and reproducible amplification.

Components	Cat.No.: A009-1	Cat. No.: AN009-2	Storage
2x AuroraNovo Realtime PCR mix R	5x 1ml	25x 1mll	-20°C, dark

This product is intended for use by trained laboratory personnel only. It is important to follow proper laboratory safety protocols when handling this product. This includes wearing appropriate protective eyewear, clothing, and gloves to reduce the risk of exposure. In the event of contact with skin or eyes, rinse immediately with copious amounts of water and seek medical attention if necessary.

Compatibility:
2x AuroraNovo Realtime PCR mix R can be used in general detection devices, such as: LineGene (Bioer Technology co., ltd.); 2x AuroraNovo Realtime PCR mix R can also be used in detection equipment using glass capillaries or passive reference, such as: LightCycler™ (Roche Molecular Systems); ABI PRISM® 7000, 7700, and 7900 (Applied Biosystems). Note: The passive reference mode of detectors should be set at "ROX".

Samples:
1. cDNA: Reverse transcription reactions from total or poly (A)+ RNA may be used directly, or after dilution, for real-time PCR. Purified cDNA by phenol/chloroform extraction and ethanol precipitation may also can be used. Oligo dT and random primers are suitable for the reverse transcription reaction.
2. Genomic DNA: Purified DNA, which would be used for general PCR, is also suitable for real-time PCR. In the case of mammalian genomic DNA, 1~10 ng genomic DNA is sufficient for real-time PCR.

Applications:
Qantitive gene expression, detection of trace DNA.

Shipping and storage:
Upon receiving the product, store the product at suggested conditions. Shipping and temporary storage for up to 5 days at room temperature has no detrimental effects on the quality of the product. Store at -20°C, protected from light; Avoid repeated freezing and thawing.

Recommended Protocol I:
Intercalation assay protocol using ABI PRISM® 7700
The following is an intercalator assay protocol to be used with ABI PRISM® 7700. For other detection devices, this protocol may require modification depending on each instruction manual.

Prepare 50µl reaction mixture:

Components	Volume
PCR grade water	16µl
2x AuroraNovo Realtime PCR mix	25µl
Primer Forward (10µM)	2µl
Primer Reverse (10µM)	2µl
DNA template	5µl
Nuclease-free water	Fill to 50µl

Notes: The primer concentration can be further optimized, if needed. The optimal range for the primers is 0.2~0.6 µM. In the case of commercially available primers, recommended conditions from those companies should be used.

Suggested qPCR program (3-step cycle):

95°C	30sec.-1 min
35-40 cycles of:	
95°C	15 sec
55-64°C	15 sec
72°C	45 sec (data collection)
Melting curve analysis	

Notes:
A. The annealing temperature in 3-step cycle should be set to 55~65°C, depending of the primer Tm value.
B. The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period. Fifteen seconds is also sufficient for denaturation during each cycle.
C. Data collection step should be longer than 30 sec.

Recommended Protocol II:
2. Intercalation assay protocol using Roche LightCycler™
The following is an intercalator assay protocol to be used with the Roche LightCycler™. In the case of other detection devices, this protocol should be modified accordingly.

Prepare 20µl reaction mixture:

Components	Volume
PCR grade water	6.4µl
2x AuroraNovo Realtime PCR mix	10µl
Primer Forward (10µM)	0.8µl
Primer Reverse (10µM)	0.8µl
DNA template	2µl
Nuclease-free water	Fill to 20µl

Notes:
The primer concentration can be further optimized, if needed. The optimal range for primers is 0.2~0.6 µM. In the case of

commercially available primers, recommended conditions from each manual should be followed.

Suggested qPCR program (3-step cycle):

95°C	30sec.-1 min
35-40 cycles of:	
95°C	5 sec
55-64°C	10 sec
72°C	15 sec (data collection)
Melting curve analysis	

Notes:

A. The annealing temperature can be set to 55~65°C, depending on the primer Tm value.

B. The annealing time should be set for 5~20 seconds. Longer annealing time results in increased efficiency, and a shorter time decreases non-specific amplification.

C. The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period. Five seconds is also sufficient for denaturation during each cycle.

D. Data collection step should be longer than 10 sec. If commercially available primers or probes are employed, the recommended conditions from each company should be used.

Technical support:

If you have any questions or concerns, please don't hesitate to reach out to our support team at support@angneovo.no. Our knowledgeable and friendly team is available to assist you and ensure a positive experience with our product.

Disclaimer And Warranty:

This product is compliant with its relevant specifications and intended for its stated purpose. However, ANGENOVO AS does not provide any other warranty or guarantee regarding the product's description or quality. Any such warranties or guarantees are explicitly excluded to the fullest extent allowed by law. ANGENOVO AS will not be held liable for any special, incidental, indirect, multiple or consequential damages related to or arising from the use of this product. The liability of ANGENOVO AS will not be limited or excluded for death or personal injury caused by its negligence, fraud, or fraudulent misrepresentation or any matter where it would be illegal to exclude or restrict such liability.

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