GE Healthcare
Life Sciences

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Reverse transcriptase-polymerase chain reaction (RT-PCR)

Ready-To-Go™ RT-PCR Beads

Description

Ready-To-Go RT-PCR Beads are stable at room temperature and designed for performing single-tube RT-PCR. The reagents are optimized for full-length cDNA synthesis to >7.5 kb and optimal sensitivity from PCR.

Applications

Ready-To-Go RT-PCR Beads can be used with either total RNA or mRNA isolated using various methods (Fig 1). RT-PCR can be applied to a variety of analyses, from detecting RNA in a species to determining relative levels of a specific RNA (Fig 2). Gene-specific PCR primers can be added at the same time as the cDNA synthesis primer to perform one-step RT-PCR, or PCR primers can be added after first-strand synthesis for two-step reactions. The beads are available in either 0.5 ml or 0.2 ml tubes—enough for 100 or 96 reactions, respectively.

Ready-To-Go RT-PCR Beads offer:

- Convenience: All the components needed for RT-PCR are included in a reaction bead. Simply add water, template RNA, and primers.
- Reproducibility: Beads are manufactured under highly controlled conditions, ensuring reproducible results (Fig 3).
- Reduced risk of contamination: Individual predispensed reactions minimize sample handling and pipetting steps, thus reducing the risk of RNA degradation, contamination, and pipetting errors.
- Reliability: Each batch of beads is function-tested to ensure quality and consistency of results.
- Ambient temperature stability: Ready-To-Go RT-PCR Beads can be shipped and stored at room temperature.

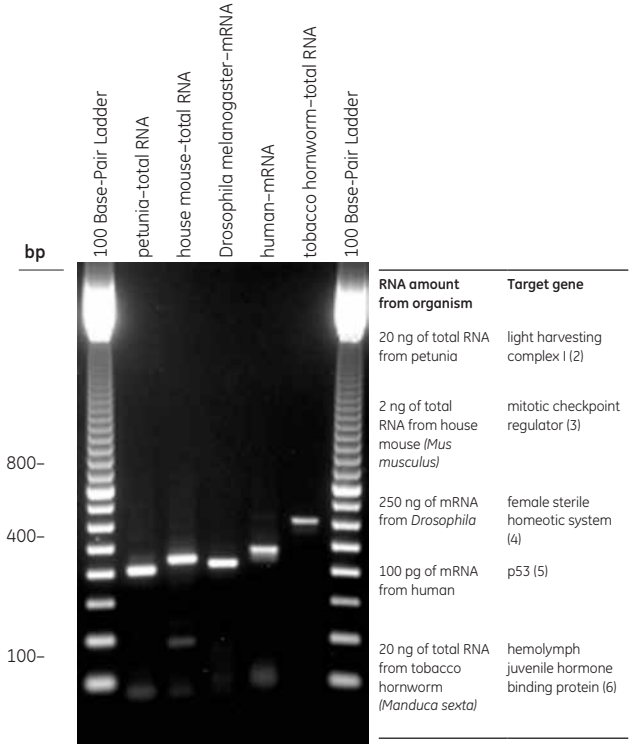

Fig 1. RT-PCR detection of specific mRNAs from a range of sources. Source organisms and RNAs are indicated above the lanes. The target genes for amplification and the amounts of RNA used are indicated in the above table.

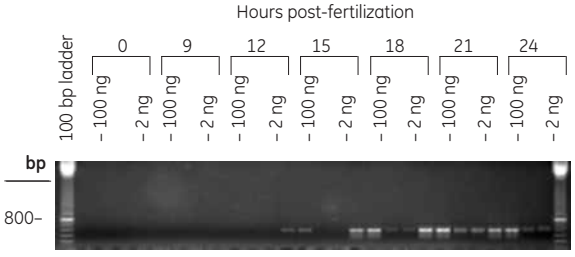

Fig 2. Relative titer of hemolymph juvenile hormone binding protein mRNA (6). Duplicate one-step RT-PCRs were run using 100 ng and 2 ng of total RNA for each time point. Five µg of $pd(N)_6$ and 10 pmol of the gene-specific primers were used for all reactions. Total RNA was isolated from a pool of 10 to 20 eggs from the black larval mutant of *Manduca sexta*.

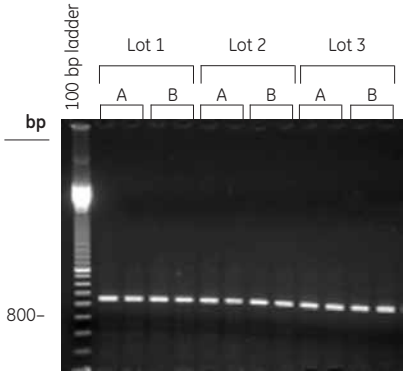

Fig 3. Reproducibility of RT-PCR using Ready-To-Go RT-PCR Beads. A 450 bp fragment from the mouse mitotic checkpoint regulator (3) gene was amplified by two researchers (A and B) using three batches of beads.

Components

RT-PCR Beads: Room-temperature stable beads containing buffer, dATP, dCTP, dGTP, dTTP, approximately 2.0 units of *Taq* DNA Polymerase, M-MuLV reverse transcriptase, RNase inhibitor, and stabilizers (including RNase/ DNase-Free BSA). When a bead is reconstituted to 50 µl, the concentration of each dNTP is 200 µM in 10 mM Tris-HCl (pH 9.0), 60 mM KCl, and 1.5 mM $MgCl_2$.

Control Mix Beads: A bead containing rabbit globin mRNA (1 ng) and 8 pmol each of 5′-specific globin primer (5′d[ACACT TCTGGTCCATCCGACTGAG]-3′) and 3′-specific globin primer (5′-d[GCCACTCACTCAGACTTTATTCAAA]-3′).

$pd(N)_6$: Lyophilized; 275 mg.

$pd(T)_{12–18}$: Lyophilized; 55 mg.

Quality Control

Each batch of Ready-To-Go RT-PCR Beads is tested to ensure its ability to generate an RT-PCR product using a Control Mix Bead and an RT-PCR bead. In addition, the beads are tested using *Drosophila melanogaster* mRNA (7.6 kb) and primers specific for a 425 bp sequence from the *fsh* gene.

Storage

At ambient temperature in pouch with desiccant.

References

1. Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159 (1987).
2. Stayton M. M. *et al.,* Characterization of a full-length petunia cDNA encoding a polypeptide of the light-harvesting complex associated with photosystem I. *Plant Mol. Biol.* **10,** 127–137 (1987).
3. Starborg M. *et al.,* A novel murine gene encoding a 216-kDa protein is related to a mitotic checkpoint regulator previously identified in Aspergillus nidulans. *J. Biol. Chem.* **269,** 24133–24137 (1994).
4. Haynes, S. R. *et al.,* The Drosophila fsh locus, a maternal effect homeotic gene, encodes apparent membrane proteins. *Dev. Biol.* **134,** 246–257 (1989).
5. Lamb, P. and Crawford, Characterization of the human p53 gene. L., *Mol. and Cell. Biol.* **6,** 1379–1385 (1986).
6. Goodman, W. G. and Chang, E.S. Juvenile hormone cellular and hemolymph binding protein, *in Comprehensive Insect Physiology, Biochemistry, and Pharmacology: Endocrinlogy I.* (Kerkut, G. A. and Gilbert, L. I., eds.) pp 491, Pergamon Press, Oxford, England (1985).

Ordering information

Product	Code number
Ready-To-Go RT-PCR Beads (0.5 ml tubes) 100 reactions	27-9266-01
Ready-To-Go RT-PCR Beads (0.2 ml tubes) 96 reactions	27-9267-01
Ready-To-Go RT-PCR Beads (0.2 ml hinged tube with cap) 96 reactions	27-9259-01

Related products

First-strand synthesis

First-Strand cDNA Synthesis Kit	27-9261-01
Ready-To-Go You-Prime First-Strand Beads	27-9264-01
Ready-To-Go T-Primed First-Strand Kit	27-9263-01

PCR

Product	Code number
PuReTaq™ Ready-To-Go PCR Beads	see gelifesciences.com
Taq DNA Polymerase	see gelifesciences.com
illustra™ Hot Start Master Mix	25-1500-01
illustra Hot Start Mix RTG	see gelifesciences.com

Nucleotides

illustra Solution dNTPs	see gelifesciences.com

mRNA and total RNA Purification

mRNA Purification Kit	27-9258-01
mRNA Purification Kit	27-9258-02
illustra RNAspin Isolation Kits	see gelifesciences.com
QuickPrep Micro mRNA Purification Kit	27-9255-01
QuickPrep mRNA Purification Kit	27-9254-01

DNA purification

ExoProStar™	see gelifesciences.com
illustra GFX™ PCR DNA and Gel Band Purification Kit	28-9034-70
MicroSpin™ S-400 HR Columns	27-5140-01
100 Base-Pair Ladder	27-4007-01

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