

GE Healthcare

Ready-To-Go You-Prime First-Strand Beads

Product Booklet

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1. Legal

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<http://www.gelifesciences.com>

GE Healthcare UK Limited.

Amersham Place,

Little Chalfont, Buckinghamshire,

HP7 9NA, UK

2. Handling

2.1. Safety warnings and precautions

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at ambient room temperature in the airtight foil pouch with the desiccant. Once opened, completely reseal the pouch, fold the sealed edge over several times and seal with a clip. In high humidity environments, store unopened and resealed pouches in a desiccator to maximize product lifetime.

3. Components of the kit

The following components are included in this product:

First-strand
reaction mix beads
(50 white tubes)

Two beads containing
buffer, dATP, dCTP, dGTP,
dTTP, Murine reverse
transcriptase (FPLCpure™),
RNAguard™ (porcine),
RNase/DNase-free BSA.

Control mix bead
(5 red tubes)

One ambient-temperature-
stable bead containing rabbit
globin mRNA (1 ng), buffer
and 8 pmol each of 5'-specific
globin primer (5'-d[ACACTTC-
TGGTCCAGTCCGACTGAG]-3')
and 3'-specific globin primer
(5'-d[GCCACTCACTCAGACTT-
TATTCAAA]-3').

Additional reagents required for the procedures are listed in
'Materials not supplied' (page 7).

4. Quality control

Each batch of Ready-To-Go™ You-Prime First-Strand Beads is tested for its ability to generate full-length cDNA molecules up to 7.5 kb. The kit is also tested for its ability to RT-PCR amplify rabbit globin mRNA using the control mix bead and AmpliTaq™.

5. Materials not supplied

Reagents

- **DEPC-treated water** — A 0.1% solution of DEPC (diethyl pyrocarbonate) in distilled water, stirred at room temperature for a minimum of 2 hours, then autoclaved.

For monitoring the cDNA synthesis reaction (optional):

- **[α -³²P]dCTP** — 10 mCi/ml, 3 000 Ci/mmol.
- **Phenol** — A saturated solution of redistilled phenol in TE buffer containing 8-hydroxy quinoline (4).
- **Chloroform/isoamyl alcohol** — Reagent-grade chloroform and isoamyl alcohol, mixed 24:1 (v/v).
- **Phenol/chloroform** — Equal parts of phenol and chloroform/isoamyl alcohol (24:1) (v/v); see above.
- **STE buffer** — 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl.

6. Introduction

Ready-To-Go You-Prime First-Strand Beads contain all the reagents necessary to generate full-length first-strand cDNA from an RNA template using a variety of primers. Kit components are provided in a room-temperature-stable format.

The reaction beads utilize Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase to generate first-strand cDNA. The conditions of this reaction have been optimized to permit full-length transcription of RNAs 7 kilobases or more in length. Each first-strand reaction requires only the addition of RNA in DEPC-treated water and a first-strand primer. The first-strand reaction may be primed with oligo(dT) primers, random primers such as pd(N)₆, or custom primers complementary to a specific mRNA sequence.

First-strand cDNA generated with the first-strand beads can be used directly as a template for PCR (i.e. RT-PCR, ref. 1-4). In this procedure, the double-stranded RNA:cDNA heteroduplex made during first-strand synthesis is heat denatured to allow the cDNA strand to be used as a template for polymerization. The specificity of the PCR amplification is based on two amplification primers which flank the cDNA segment to be amplified and hybridize to complementary strands. Repeated cycles of denaturation, primer annealing, and primer extension by *Taq* DNA polymerase* can result in exponential amplification of a target cDNA. Even cDNA made from relatively rare transcripts can be successfully amplified using this technique.

* Subject to any restriction by patents owned by Roche

Alternatively, the completed first-strand cDNA can be used in standard Gubler-Hoffman second-strand cDNA synthesis (5). Following synthesis of the first-strand cDNA, RNase H is used to nick the RNA strand of the RNA:cDNA heteroduplex. DNA polymerase I then uses these nicks to replace the RNA with DNA by nick translation.

Ready-To-Go You-Prime First-Strand Beads are manufactured by a proprietary technology licensed to GE Healthcare†. Each kit contains predispensed first-strand reaction mixes which are treated by a special process which turns each one into two small beads, stable at ambient temperatures. Each first-strand reaction is quickly reconstituted when mixed with an aqueous solution. Thus with Ready-To-Go You-Prime First-Strand Beads, preparation and setup are convenient: The beads are reconstituted with the denatured RNA sample, primer is added, and the reaction is placed at 37°C for 60 minutes. The completed first-strand reaction is then available for PCR amplification or second-strand cDNA synthesis.

7. Protocols

7.1. Essential preliminaries

The use of intact, undegraded RNA is of primary importance to the success of first-strand cDNA synthesis. We strongly recommend the use of our mRNA purification kits for preparing high-quality mRNA. For rapid purification of mRNA directly from cells or tissues, we recommend either *illustra™ QuickPrep™ Micro* mRNA Purification Kit (27-9255-01) or the original *illustra QuickPrep* mRNA Purification Kit (27-9254-01).

The *illustra QuickPrep Micro* mRNA Purification Kit can purify mRNA in 15 minutes from samples ranging in size from a single cell to 0.1 g of tissue; it contains sufficient materials for 24 samples. The *illustra QuickPrep* mRNA Purification Kit can purify mRNA in less than 1 hour from samples ranging in size from a single cell to as much as 0.5 g of tissue; it contains sufficient materials for four samples. For samples available in greater quantity, or having high RNase activity, we suggest the combined application of the RNA Extraction Kit and the mRNA Purification Kit.

For purification of total RNA we recommend the use of the *illustra RNAspin* kit range (see page 18 for ordering information). With these kits total RNA from cells and tissues can be extracted and purified for use in downstream applications like RT-PCR.

If the cDNA portion of the heteroduplex is to be used directly as template in Gubler-Hoffman second-strand cDNA synthesis, we recommend using cloned *E. coli* Ribonuclease H and DNA Polymerase I. For both second-strand cDNA synthesis and PCR, we recommend the DNA Polymerization Mix as the source of ultrapure deoxyribonucleotides.

Cautions

- Each tube of Ready-To-Go reaction mix beads (white tube) contains two beads. Two beads are required for a complete first-strand reaction. Do not use only one bead.
- Wear gloves to avoid contamination with ribonucleases from the skin.
- When performing PCR, exercise extreme care to prevent DNA contamination. Always use sterile pipette tips and microcentrifuge tubes, and avoid carry-over contamination of stock solutions.

Control mix

The five control mixes included in the kit are packaged in red 0.5 ml microfuge tubes. Each contains one room-temperature-stable bead containing 1 ng of rabbit globin mRNA and 8 pmol each of two globin-specific PCR primers. A control mix bead can be used to evaluate the performance of the first-strand reaction and the PCR amplification by adding the rehydrated control mix to a tube of first-strand reaction mix beads and performing RT-PCR.

The globin primer specific for the 3' end of the globin message will anneal to the mRNA and serve as a primer for the reverse transcriptase to produce first-strand globin cDNA. Subsequent PCR amplification of the first-strand product utilizes the 5' globin-specific primer and the remaining 3' globin-specific primer as PCR primers, resulting in a 550 bp PCR product.

Additional reagents required for the procedures are listed in 'Materials not supplied' (page 7).

7.2. First-strand cDNA synthesis

The amount of RNA needed in the first-strand reaction will vary depending on the downstream application and the relative abundance of the message of interest: For PCR, we recommend using 20 ng–2 µg of mRNA or 100 ng–5 µg of total RNA. For

Gubler-Hoffman second-strand synthesis, we recommend starting with 1–5 µg of mRNA.

1. Bring the RNA sample (or the control mix bead) to a volume of 25–30 µl in an RNase-free microcentrifuge tube using DEPC-treated water.
2. Heat at 65°C for 10 minutes, then chill on ice for 2 minutes. At the same time, check that two beads are visible in the bottom of the tube of first-strand reaction mix beads (white tube). If necessary, tap the tube against a hard surface to bring the beads to the bottom.
3. Transfer the RNA solution (or the control mix solution) to the tube of first-strand reaction mix beads. Do not mix.
4. Add chosen primer (see Table 1) and DEPC-treated water to a final volume of 33 µl. Let sit at room temperature for approximately 1 minute.
5. Mix the contents of the tube by gently vortexing, or by repeatedly pipetting the mixture up and down. Centrifuge briefly to collect the contents at the bottom of the tube.
6. Incubate at 37°C for 60 minutes.

The completed first-strand reaction is now ready for immediate PCR amplification or second-strand cDNA synthesis.

Table 1. Recommended quantities of primer for first-strand synthesis.

First-strand cDNA primer	Intended application	
	Second-strand synthesis	PCR-amplified synthesis
pd(N)6	0.2–0.02 µg	0.2 µg
Oligo(dT)	5 µg	0.5 µg
Specific primer	40–400 pmol	20–40 pmol

7.3. PCR amplification conditions

The volume of the first-strand cDNA reaction used for amplification will depend on the relative abundance of the cDNA of interest in the final reaction mixture. For cDNAs from very rare messages, we recommend using the entire first-strand reaction for amplification. For more abundant messages, you may be able to use less. However, if you use less than 10 μ l of the first-strand reaction, it will be necessary to adjust the amplification reaction conditions according to Table 2 (page 14).

The buffer and dNTP conditions in the completed first-strand cDNA reaction are 50 mM Tris (pH 8.3), 75 mM KCl, 7.5 mM DTT, 10 mM $MgCl_2$, 0.08 mg/ml BSA and 2.4 mM each dNTP.

When amplifying the entire first-strand reaction (33 μ l), you will need to add only the PCR primers and the *Taq* DNA polymerase. When amplifying less than 10 μ l of the first-strand reaction, you will also need to add PCR buffer [10 \times PCR buffer is 500 mM KCl, 100 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$] and dNTP mix (e.g., DNA Polymerization Mix from GE Healthcare).

The following table can be used as a guideline when amplifying various amounts of the first-strand reaction. For the control reaction, the entire 33 μ l should be amplified. Because both PCR primers are already present in the control reaction, only the addition of water (66 μ l) and *Taq* DNA polymerase (1 μ l) is required.

Table 2. Recommended PCR conditions for various amounts of first-strand cDNA Synthesis reaction.

Volume of cDNA rxn	10 × PCR buffer	20 mM dNTP mix	Upstream primer (10–40 pmol)	Downstream primer (10–40 pmol)	Water	Taq pol
1–5 µl	10 µl	1 µl	x µl	x µl	to 100 µl	2.5 u
6–10 µl	10 µl	0	x µl	x µl	to 100 µl	2.5 u
33 µl	0	0	x µl	x µl	to 100 µl	2.5 u

1. Mix the appropriate volumes of reagents (see Table 2) in a sterile 0.5 ml microcentrifuge tube and layer mineral oil over the sample to prevent evaporation (if required by the thermal cycler).
2. Place the samples in a thermal cycler and cycle 20–50 times depending on the abundance of the target. For the control reaction we recommend the following cycle profile: denaturation at 95°C for 1 minute, 62°C for 1 minute, and polymerization at 72°C for 1 minute. Repeat for a total of 32 cycles.

8. Appendix

8.1. Monitoring first-strand synthesis

The easiest way to monitor the activity of the first-strand reaction is to use one of the control mixes provided with the kit, proceed through PCR, and look for the presence of the 550 bp globin PCR product on an agarose gel. An alternative method for monitoring first-strand cDNA synthesis is to incorporate radioactive nucleotides during first-strand synthesis, separate the reaction products by gel electrophoresis, and analyze their size by autoradiography. In order to assay first-strand cDNA synthesis with this method, follow the directions below using 1–5 µg of your own RNA.

- Heat 25–30 µl of an RNA solution (1–5 µg) to 65°C for 10 minutes, then chill on ice for 2 minutes.
- Transfer the RNA solution to a tube of first-strand reaction mix beads and add 20 µCi of [α -³²P]dCTP (see 'Materials not supplied'), primer and DEPC-treated water to a final volume of 33 µl. Do not mix.
- Let sit at room temperature for approximately 1 minute then mix the contents of the tube by gently vortexing or by repeatedly pipetting the mixture up and down. Centrifuge briefly to collect the contents at the bottom of the tube.
- Incubate at 37°C for 60 minutes.
- Bring the final volume to 100 µl by adding 65 µl of STE buffer (prepared by the researcher) and transfer to a fresh 1.5 ml microfuge tube. Add 100 µl of phenol/chloroform, vortex, then centrifuge for 1 minute.
- Collect the upper (aqueous) phase and remove any unincorporated radioactive nucleotides by either spun-column

chromatography (e.g. using illustra ProbeQuant™ G-50 Micro Columns from GE Healthcare) or standard ethanol precipitation.

- Using established procedures (6), analyze 50–100 μl of the sample by electrophoresis and autoradiography of a 1% agarose gel.

9. References

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3. Chelley et al., *Nature* **333**, 858 (1988).
4. Rappolee et al., *Science* **241**, 708 (1988).
5. Gubler, U. and Hoffman, B. J., *Gene* **25**, 263 (1983).
6. Maniatis, T., Fritsch, E. F. and Sambrook, J., *Molecular Cloning: A laboratory manual*, Cold Spring Harbor Laboratory, 1982.

10. Companion products available from GE Healthcare

Product	Pack size	Product number
illustra QuickPrep Micro mRNA Purification Kit (24 purifications)	1 kit	27-9255-01
illustra QuickPrep mRNA Purification Kit (4 purifications)	1 kit	27-9254-01
DNA Polymerization Mix	10 μ mol of each dNTP	28-4065-57
(solution)	40 μ mol of each dNTP	28-4065-58
Taq DNA Polymerase (cloned)	250 units	27-0798-04
	4 \times 250 units	27-0798-05
	10 \times 250 units	27-0798-06
Taq DNA Polymerase	250 units	27-0799-04
	4 \times 250 units	27-0799-05
	10 \times 250 units	27-0799-06
	1000 units	27-0799-61
	5000 units	27-0799-62
	10 000 units	27-0799-63
	25 000 units	27-0799-64
illustra Microspin™ S-200 HR Columns	50 columns	27-5120-01
illustra Microspin S-300 HR Columns	50 columns	27-5130-01

Product	Pack size	Product number
illustra Microspin S-400 HR Columns	50 columns	27-5140-01
illustra ProbeQuant G-50 Micro Columns	50 columns	28-9034-08
illustra RNAspin Mini Isolation Kit	20 preps	25-0500-70
	50 preps	25-0500-71
	250 preps	25-0500-72
illustra RNAspin Midi Isolation Kit	20 preps	25-0500-73
illustra RNAspin 96 RNA Isolation Kit	2 × 96 preps	25-0500-74
	4 × 96 preps	25-0500-75
	24 × 96 preps	25-0500-76
illustra mRNA Purification Kit	2 purifications	27-9258-01
	4 purifications	27-9258-02
Amersham™ Hyperfilm™ MP		
18 × 24cm	50 sheets	28-9068-43
	100 sheets	28-9068-44
24 × 30cm	50 sheets	28-9068-47
35 × 43cm	50 sheets	28-9068-48
5 × 7"	50 sheets	28-9068-42
8 × 10"	50 sheets	28-9068-45
8 × 10"	100 sheets	28-9068-46
18 × 24 cm, enveloped	50 sheets	28-9068-50

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For contact information for your local office,
please visit: <http://www.gelifesciences.com/contact>

GE Healthcare UK Limited
Amersham Place
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

<http://www.gelifesciences.com>



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