Certificate of Analysis

ImProm-II™ Reverse Transcriptase

Part# 9PIA380

Revised 10/16

Cat.#	Size
A3801	10 reactions
A3802	100 reactions
A3803	500 reactions

Description: ImProm-II™ Reverse Transcriptase[a] is provided in quantities sufficient for 10–500 first-strand cDNA synthesis reactions of 20µl each. **Additional components are required to perform the reactions.** Contains one of the following:

Cat.# A3801

Part No.	Component	Size
M314B	ImProm-II™ Reverse Transcriptase, 1µl/reaction	10µl
M289B	ImProm-II™ 5X Reaction Buffer	100µl
A351B	$MgCl_2$, 25mM	750µl

Cat.# A3802

Part No.	Component	Size
M314A	ImProm-II™ Reverse Transcriptase, 1µl/reaction	100µl
M289A	ImProm-II™ 5X Reaction Buffer	600µl
A351H	$MgCl_2$, 25mM	1.2ml

Cat.# A3803

Part No.	Component	Size
M314C	ImProm-II™ Reverse Transcriptase, 1µl/reaction	500µl
M289C	ImProm-II™ 5X Reaction Buffer	2 × 1,250µl
A351H	$MgCl_2$, 25mM	3 × 1.2ml

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

Usage Note: Additional components are required to perform RT-PCR. These include: appropriate primers, nuclease-free water, dNTPs and *Taq* DNA polymerase. Recombinant RNasin® Ribonuclease Inhibitor is optional but recommended.

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Promega

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Quality Control Assays

Activity Assays

First-Strand cDNA Synthesis: Two hundred units of enzyme are used to produce cDNA from 1µg of 1.2kb and 6.5kb control RNAs in separate reactions, using [^{32}P] dCTP as a tracer. The minimum specification is 120ng of first-strand cDNA made from 1µg of RNA. The cDNA product must be >90% full length as determined by gel electrophoresis and autoradiography.

Amplification: When 0.25 zeptomoles (approximately 100 copies) of 1.2kb Kanamycin Positive Control RNA (Cat.# C1381) is reverse transcribed at 42°C and amplified, the result is a clear, discrete 323bp DNA product as visualized on an agarose gel by ethidium bromide staining.

Signed by:

R. Wheeler, Quality Assurance

[a]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

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Printed in USA. Revised 10/16.

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Usage Information

I. Standard Reverse Transcription Protocol

A. Prepare RNA Target and Primer

1. Use sterile, nuclease-free, thin-walled tubes, prechilled on ice.
2. For each 20µl reverse transcription (RT) reaction, combine:

Component	Volume
RNA template	up to 1µg
primer	20pmol or 0.5µg
nuclease-free water to a final volume of	5µl

3. Incubate at 70°C for 5 minutes.
4. Quick-chill at 4°C for 5 minutes and hold on ice.

Notes:

- RT reactions of 20µl process up to 1µg of total RNA, mRNA or synthetic transcript, and allow detection of 100 copies of a specific RNA target.
- Primer amounts recommended for efficient cDNA synthesis are 0.5µg of oligo(dT) or random primers, or 20pmol of gene-specific primers per 20µl reaction.
- For best results, heat denature the primers and target in a controlled-temperature heat block; then chill immediately in an ice water bath for at least 5 minutes. Hold on ice until addition to RT reaction.

B. Prepare Reverse Transcription Mix

1. For each 20µl RT reaction, combine

Component	Final Concentration	Volume
nuclease-free water		Xµl
ImProm-II™ 5X Reaction Buffer	1X	4µl
$MgCl_2$, 25mM*	3mM*	2.4µl
dNTP mix* (10mM each dNTP)	0.5mM*	1µl
Recombinant RNasin® Ribonuclease Inhibitor	1u/µl	20u
Vortex the mixture. Add:		
Improm-II™ Reverse Transcriptase		1µl
Final Volume RT Mix per 20µl reaction		15µl

*See notes below

2. Vortex gently to mix.
3. Dispense 15µl aliquots into reaction tubes.

Notes:

- Combine indicated reagents in the order specified in a sterile, nuclease-free reaction tube.
- $MgCl_2$ may be optimized between 1.5–8.0mM.
- Final dNTP concentration is 0.5mM.
- Use of Recombinant RNasin® Ribonuclease Inhibitor is optional but recommended.
- We recommend keeping the RT reaction mix chilled on ice prior to incubation.

C. Add Template + Primers to the Reaction Mix

1. For each individual reaction, add 5µl of the appropriate Template + Primer mix to the 15µl reverse transcription or RT-PCR mix. If necessary, overlay with nuclease-free mineral oil. The final volume for each individual reaction will be 20µl.

Notes:

- Add the RNA Template + Primer mix immediately prior to incubation.
- Keep tubes closed whenever possible to avoid contamination, and be careful to avoid cross contamination.

D. Reverse Transcription

1. Anneal at 25°C for 5 minutes.
2. Extend the first strand for 60 minutes at 42°C. The extension temperature may be optimized between 37–55°C.
3. Heat-inactivate the ImProm-II™ Reverse Transcriptase by incubating at 70°C for 15 minutes.
4. Analyze cDNA, proceed with PCR or store frozen.

Notes:

- Place the reaction tubes in a controlled-temperature heating block.
- Annealing conditions may require optimization. See Technical Manual #TM236 for details regarding optimization.
- First-strand cDNA synthesis occurs during the extension step. The extension temperature may require optimization. See Technical Manual #TM236 for details regarding optimization.

E. PCR Amplification (Optional)

1. The cDNA may be amplified by adding the products of the heat inactivated reverse transcription reaction directly to the PCR mix and proceeding with thermal cycling.
2. Prepare PCR Mix, minus the cDNA sample by combining the amplification reagents in a sterile 1.5ml microcentrifuge tube on ice. Combine the components in the order listed, vortex gently to mix, and keep on ice.

Component	Final Concentration	Volume per 100µl reaction (20µl RT reaction)
nuclease-free water		Xµl
10X thermophilic polymerase reaction buffer (without $MgCl_2$)*	1X	8.0µl
$MgCl_2$, 25mM	2mM*	5.6µl
dNTP mix, 10mM	0.2mM	1µl
upstream primer	1µM	Xµl
downstream primer	1µM	Xµl
Taq DNA polymerase	5.0u	1.0µl
PCR Mix volume per reaction		80µl
Volume of RT reaction added		20.0µl
Total PCR Volume		100.0µl

*For experimental systems, Mg^{2+} should be optimized between 1.5–2.5mM.

3. Dispense the appropriate volume of PCR mix into each chilled reaction tube.
4. Add the appropriate aliquot of the reverse transcription reaction to the PCR mix.
5. If necessary, overlay the reaction with nuclease-free mineral oil and proceed with amplification program.

Notes:

- In the table of PCR component additions shown, the amounts of 10X Reaction Buffer, 25mM $MgCl_2$ and dNTP mix combined in the PCR amplifications supplement the specified volume of reverse transcription reaction that is added. If the $MgCl_2$ concentration of the RT reaction is changed, these proportions will require modifications.
- **The volume of the PCR amplification may be scaled for volumes less than 100µl. Carryover concentrations of $MgCl_2$, dNTPs, buffer and primers must be considered when calculating the volumes of the PCR mix components.**

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Printed in USA. Revised 10/16.