

FirstChoice® PCR-Ready Human Small Intestine cDNA

Store at -20°C.

Do not store in a frost-free freezer.

Catalog #:	AM3335
Amount:	Approximately 50 reactions
Concentration:	0.5 ng/μL
Volume:	50 μL
Additional Materials Included:	Control Primer Mix, 5 μM, 20 μL (control primers target a constitutive housekeeping gene)
Storage Conditions:	Store at -20°C. <i>Do not store in a frost-free freezer.</i>
Storage Buffer:	10 mM Tris, pH 8.0, 0.1 mM EDTA

USER INFORMATION

Product Description:

Ambion's FirstChoice® PCR-Ready cDNA is prepared from the highest possible quality, DNA-free RNA that has been triple-oligo(dT) selected to maximize the mRNA content of each preparation. Each cDNA is made up of equal mass amounts of the products of two separate reverse transcription reactions using Ambion's ArrayScript™ Reverse Transcriptase (Cat #AM2048), one primed with oligo (dT), one with random decamers. This ensures the best possible representation of the original mRNA population. After synthesis, the cDNA is treated with RNase A and RNase T1 to degrade the RNA template, and is purified by size-exclusion chromatography to remove unincorporated nucleotides, primers, and small (<100 nt) reaction products. The cDNA is quantified using the NanoDrop® 1000A Spectrophotometer and adjusted to 0.5 ng/μL with 10 mM Tris pH 8.0, 0.1 mM EDTA. The size distribution of the cDNA is determined on the Agilent® 2100 bioanalyzer. Finally, the cDNA preparation is performance-tested in a polymerase chain reaction (PCR) using the included Control Primer Mix (see the control reaction protocol following).

All FirstChoice Human cDNAs are synthesized from RNA isolated from human tissues that have tested negative for the following: HIV-1, HIV-2, HTLV-1 (human T-cell lymphocytotropic virus 1), RPR (from syphilis), HbsAg (hepatitis B surface antigen), Anti-HBL Hepatitis B, and HCV [1].

Tissue Procurement:

Ambion is committed to the highest ethical standards for tissue procurement.

- Ambion is committed to maintaining the highest level of ethical standards with regard to all tissue that is used in the preparation of RNA and RNA-derived products.
- Ambion certifies that all of our human-derived materials have been prepared from tissue obtained with consent from a fully informed donor or a member of the donor's family.
- Ambion further guarantees that all human tissue received has been procured following our internal guidelines and policies.

Note: No known test method can offer absolute assurance that products derived from human biological materials will not transmit infectious diseases. Employ appropriate universal precautions when using human-derived materials [1].

Applications:

These cDNAs can be used for gene discovery or expression analysis applications. 1 μL of FirstChoice cDNA contains 0.5 ng of first-strand cDNA. This is roughly equivalent to the cDNA produced from 50 ng of total RNA in a standard reverse transcription reaction. Several factors, including target abundance and PCR primer efficiency, will determine how much FirstChoice PCR-Ready cDNA to use in PCRs, but typically 0.5 ng is a good starting point. Using 0.5 ng per PCR, the 25 ng of cDNA supplied is enough for 50 typical PCRs. To detect a rare transcript, more cDNA may be required. Conversely, for abundant transcripts less cDNA may be needed.

Control Reaction Protocol

Setup

PCR-Ready cDNA	1 μL
10X PCR Buffer	5 μL
2.5 mM dNTP	4 μL
Control Primer Mix (5 μM)	4 μL
Nuclease-free water	35.75 μL
Taq Polymerase	0.25 μL

PCR Thermal Cycling Conditions

	Stage	Reps	Temp	Time
Initial Denaturation	1	1	94°C	3 min
Amplification	2	35	94°C	30 sec
			60°C	30 sec
			72°C	1 min
Final Extension	3	1	72°C	7 min
Hold	Hold	n/a	4°C	n/a

Analysis

Run 10 µL of the PCR product on a 2% agarose gel containing 1 µg/mL ethidium bromide. There should be one major band of 294 bp from this control reaction.

- Reference:**
1. Grizzle WE and Polt SS (1998) Guidelines to avoid personnel contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. **11**:191–199.

QUALITY CONTROL

Nonspecific Endonuclease Activity: Meets or exceeds specification when a sample is incubated for 14–16 hr with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease Activity: Meets or exceeds specification when a sample is incubated for 14–16 hr with 40 ng ³²P-labeled *Sau3A* fragments of pUC19 and analyzed by PAGE.

Functional Testing: The PCR-Ready cDNA is functionally tested with the Control Primer Mix in the recommended PCR reaction.

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

Warranty and Liability: *For research use only. Not for use in diagnostic procedures.*

Ambion is committed to providing the highest quality reagents at competitive prices. Ambion warrants that for the earlier of (i) one (1) year from the date of shipment or (ii) until the shelf life date, expiration date, "use by" date, "guaranty date" or other end-of-recommended-use date stated on the product label or in product literature that accompanies shipment of the product, Ambion's products meet or exceed the performance standards described in the product specification sheets if stored and used properly. No other warranties of any kind, expressed or implied, are provided by Ambion. WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE ARE EXPRESSLY DISCLAIMED. AMBION'S LIABILITY SHALL NOT EXCEED THE PURCHASE PRICE OF THE PRODUCT. AMBION SHALL HAVE NO LIABILITY FOR INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES ARISING FROM THE USE, RESULTS OF USE, OR INABILITY TO USE ITS PRODUCTS. See the full limited warranty statement that accompanies products for full terms, conditions and limitations of Ambion's limited product warranty, or contact Ambion for a copy.

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