## Recommendations for Optimal Results Using the iScript Select cDNA Synthesis Kit

The maximum amount of the cDNA reaction that is recommended for downstream PCR is one-tenth of the reaction volume, typically 2 µl.

When using larger amounts of input RNA (>1 µg), the reaction should be scaled up; e.g., 40 µl reaction for 2 µg, or 100 µl reaction for 5 µg to ensure optimum synthesis efficiency.

## Related Amplification Products From Bio-Rad Laboratories

### Reagents for PCR or Real-Time PCR

| | |
|---|---|
| iTaq™ DNA Polymerase | 170-8870 |
| iQ™ Supermix | 170-8860 |
| iQ SYBR® Green Supermix | 170-8880 |
| iTaq Supermix With ROX | 170-8854 |
| iTaq SYBR Green Supermix With ROX | 170-8850 |
| iScript cDNA Synthesis Kit | 170-8890 |
| iScript One Step RT-PCR Kit With SYBR Green | 170-8892 |
| iScript One Step RT-PCR Kit for Probes | 170-8894 |
| iProof™ High-Fidelity DNA Polymerase | 172-5301 |

### Other Reagents

| | |
|---|---|
| dNTP Mix, 200 µl, 10 mM each dNTP (dATP, dCTP, dGTP, dTTP) | 170-8874 |
| $MgCl_2$ Solution, 50 mM, 1.25 ml | 170-8872 |

### Aerosol Barrier Pipet Tips

| | |
|---|---|
| Xcluda™ Style B Tips | 211-2006 |

### Nuclease-Free Tubes

| | |
|---|---|
| 0.2 ml Thin-Wall Tubes | TWI-0201 |
| 0.2 ml Thin-Wall Plates | HSP-9601 (Low-Profile)<br>HSS-9601 (Full Height) |

### RNA Purification Kits

| | |
|---|---|
| Aurum™ Total RNA Mini Kit | 732-6820 |
| Aurum Total RNA Kit, 2 x 96-well | 732-6800 |
| Aurum Total RNA Fatty and Fibrous Tissue Kit | 732-6830 |
| PureZOL™ RNA Isolation Reagent | 732-6890 |

**For ordering information on larger pack sizes, or to learn more about Bio-Rad amplification reagents and instruments, visit www.bio-rad.com/amplification/**



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10001023 Rev B

BIO-RAD

# iScript™ Select cDNA Synthesis Kit

| | |
|---|---|
| 25 x 20 µl reactions | 170-8896 |
| 100 x 20 µl reactions | 170-8897 |

For research purposes only
Store at -20°C

The iScript Select cDNA synthesis kit is a sensitive, flexible, and easy-to-use kit for the generation of first-strand cDNA. This robust reverse transcription kit allows a selection of first-strand priming strategies†, oligo(dT) primers only, random primers only, or user-designed gene-specific primers.

This cDNA synthesis kit provides all required reagents, except RNA template and gene-specific primers, to create first-strand cDNA from an RNA template. All kit components are optimized to facilitate efficient cDNA synthesis using 1 pg to 1 µg of input total RNA.

The iScript reverse transcriptase mixture contains a recombinant RNase H+ MMLV reverse transcriptase preblended with a recombinant RNase inhibitor. The activity of this reverse transcriptase is optimized for use with the 5x iScript select reaction mix. This unique blend of buffers, stabilizers, and dNTPs streamlines reaction setup and ensures robust synthesis of first-strand cDNA.

To enhance first-strand priming, the iScript select cDNA sythesis kit incorporates a proprietary enhancer solution into the primer-template hybridization step. To simplify reaction setup, this enhancer is preblended with the oligo(dT) primers and random primers provided in the kit. Consequently, there is no need to add enhancer solution to reactions using the provided primer mixes. However, when using a gene-specific primer (GSP), the enhancer solution must be added to the reaction. A separate protocol and tube labeled *GSP Enhancer Solution* is included for this purpose. The addition of enhancer solution to cDNA reactions can significantly improve yields, resulting in earlier detection in real-time PCR.

## Storage and Stability

Store kit components at –20°C in a constant-temperature freezer. When stored under these conditions, kit components are stable for a minimum of one year after the shipping date. Nuclease-free water can be stored at room temperature.

† To perform first-strand synthesis with a mixture of random primers and oligo(dT) primers, the iScript cDNA synthesis kit (170-8890 or 170-8891) with an optimized, preblended mixture of random primers and oligo(dT) primers is recommended.

## Kit Contents

| Reagent | 25 Reactions Volume | 100 Reactions Volume | Description |
|---|---|---|---|
| iScript reverse transcriptase | 25 µl | 100 µl | RNase $H^+$ MMLV reverse transcriptase and RNase inhibitor protein |
| 5x iScript select reaction mix | 400 µl | 400 µl | 5x reaction buffer containing dNTPs, magnesium chloride, and stabilizers |
| Oligo$(dT)_{20}$ primer mix | 200 µl | 200 µl | Purified oligo$(dT)_{20}$ primer in a proprietary enhancer solution |
| Random primer mix | 200 µl | 200 µl | Purified random primers in a proprietary enhancer solution |
| GSP enhancer solution | 200 µl | 200 µl | Proprietary solution for reactions using gene-specific primers |
| Nuclease-free water | 1.5 ml | 1.5 ml | |

## Reaction Setup With Oligo(dT) Primers or Random Primers

### Important Note – Please Read Before Starting

This protocol is for use with either oligo(dT) or random primers. Only use the provided primers. Use of primers from other sources can adversely affect performance and sensitivity. To use gene-specific primers, please follow the protocol *Reaction Setup With Gene-Specific Primers*.

### Protocol for Oligo(dT) or Random Primers

1. Thaw all components except iScript reverse transcriptase. Mix thoroughly and briefly centrifuge to collect contents to the bottom of the tube before using. Place components on ice.
2. Add the following components to a 0.2 ml PCR tube or each well of a 96-well PCR reaction plate on ice:

| Components | Volume |
|---|---|
| Nuclease-free water | Variable |
| 5x iScript select reaction mix | 4 µl |
| Oligo$(dT)_{20}$ primer or random primer | 2 µl |
| RNA sample (1 pg to 1 µg total RNA) | Variable |
| iScript reverse transcriptase | 1 µl |
| Total | 20 µl |

   *Note: for multiple reactions, prepare a master mix with the above components, except RNA, and then dispense to each reaction*.

3. Mix gently and incubate as follows:

   For oligo(dT)-primed cDNA reactions, incubate for 60–90 min at 42°C.
   For random-primed cDNA reactions, incubate for 5 min at 25°C, then 30 min at 42°C.
4. Incubate at 85°C for 5 min to heat-inactivate the reverse transcriptase.
5. Store cDNA product at –20°C to +4°C.
6. The resulting cDNA product can be used directly for PCR amplification. Typically, one-tenth (2 µl) of the first-strand reaction provides sufficient target for most PCR applications. Optionally, the cDNA can be diluted in TE buffer [10 mM Tris (pH 8.0), 0.1 mM EDTA] for addition of larger volumes (5–10 µl) to PCR reactions.

## Reaction Setup With Gene-Specific Primers

### Important Note – Please Read Before Starting

This protocol is for use with user-defined gene-specific primers. For random or oligo(dT) primers, please follow the protocol *Reaction Setup With Oligo(dT) Primers or Random Primers*.

### Protocol for Gene-Specific Primers

1. Thaw all components except iScript reverse transcriptase. Mix thoroughly and briefly centrifuge to collect contents to the bottom of the tube before using. Place components on ice.
2. Add the following components to a 0.2 ml PCR tube or each well of a 96-well PCR reaction plate on ice:

| Components | Volume | |
|---|---|---|
| Nuclease-free water | Variable | |
| 5x iScript select reaction mix | 4 µl | |
| Gene-specific primer (2–10 pmol) | Variable | (100–500 nM in 20 µl final volume) |
| GSP enhancer solution | 2 µl | |
| RNA sample (1 pg to 1 µg total RNA) | Variable | |
| iScript reverse transcriptase | 1 µl | |
| Total | 20 µl | |

   *Note: for multiple reactions, prepare a master mix with the above components, except RNA, and then dispense to each reaction*.

3. Mix gently and incubate at 42°C for 30–60 min.
   *As required, incubation times can be extended to create longer cDNAs for cloning purposes*.
4. Incubate at 85°C for 5 min to heat-inactivate the reverse transcriptase.
5. Store cDNA product at –20°C to +4°C.
6. The resulting cDNA product can be used directly for PCR amplification. Typically, one-tenth (2 µl) of the first-strand reaction provides sufficient target for most PCR applications. Optionally, the cDNA can be diluted in TE buffer [10 mM Tris (pH 8.0), 0.1 mM EDTA] for addition of larger volumes (5–10 µl) to PCR reactions.