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
iScript 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 172-5901

Other Reagents

dNTP Mix, 200 µl, 10 mM each dNTP 170-8874
(dtATP, dCTP, dGTP, dTTP)
MgCl₂ 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)
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/

Bio-Rad Laboratories, Inc. is licensed by Molecular Probes, Inc. to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only. SYBR Green is a registered trademark of Molecular Probes, Inc.

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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

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

### 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:

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free water</td>
<td>Variable</td>
</tr>
<tr>
<td>5x iScript select reaction mix</td>
<td>4 µl</td>
</tr>
<tr>
<td>Oligo(dT)$_{20}$ primer or random primer</td>
<td>2 µl</td>
</tr>
<tr>
<td>RNA sample (1 pg to 1 µg total RNA)</td>
<td>Variable</td>
</tr>
<tr>
<td>iScript reverse transcriptase</td>
<td>1 µl</td>
</tr>
<tr>
<td>Total</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

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.
4. Incubate at 85°C for 5 min to heat-inactivate the reverse transcriptase.
5. Store cDNA product at −20°C to +4°C.

### 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:

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

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.