iScript™ gDNA Clear cDNA Synthesis Kit

Catalog #	Description
172-5034	**iScript gDNA Clear cDNA Synthesis Kit**, 25 x 20 µl reactions
172-5035	**iScript gDNA Clear cDNA Synthesis Kit**, 100 x 20 µl reactions

For research use only.

Introduction

The iScript gDNA Clear cDNA Synthesis Kit is a sensitive, fast, and convenient reagent for gene expression analysis and real-time quantitative PCR (qPCR). The preblended 5x iScript Reverse Transcription Supermix contains all the necessary components, except RNA template, in one tube for first-strand cDNA synthesis. Specially formulated DNase reagents are also included for removing genomic DNA (gDNA) contamination in the RNA sample prior to cDNA synthesis.

- **Accuracy and confidence** — includes specially formulated DNase and DNase buffer solutions to eliminate gDNA from influencing expression data
- **Data reproducibility** — 1-tube reverse transcription (RT) supermix format reduces pipetting steps and promotes consistent and reproducible results
- **Simple and fast** — short protocol (36 min) and 1-tube RT supermix format allow easy cDNA synthesis reaction setup and fast results
- **Broad dynamic range** — works with a broad linear dynamic range of input total RNA (1 µg–1 pg) and allows sensitive detection of target genes with low expression levels
- **Primer design flexibility** — features an optimum blend of oligo(dT) and random primers to provide unbiased representation of the 5' and 3' regions of target genes for flexibility in qPCR primer design

Storage and Stability

Guaranteed for 12 months when stored as indicated in Table 1. Components will not freeze at recommended temperatures.

Table 1. Kit contents.

Reagent	Description	Storage, °C
iScript Reverse Transcription Supermix	5x RT supermix with RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase, dNTPs, oligo(dT), random primers, and RNase inhibitor	–20
iScript No-RT Control Supermix	5x no-RT control supermix, contains all components of iScript Reverse Transcription Supermix except reverse transcriptase	–20
iScript DNase	Concentrated custom DNase I solution	–20
iScript DNase Buffer	Concentrated proprietary DNase buffer solution	4
Nuclease-Free Water	—	–20

Reaction Setups

For optimal results, reactions should be assembled on ice using sterile and nuclease-free tubes, tube strips, or plates. The workflow in Figure 1 illustrates reaction setups.

ℹ If multiple reactions are required, scale up appropriately. However, please note that the kit component volumes provided do not take into account the preparation of excess master mix.

Reaction Setup for Removing Genomic DNA

1. Make a DNase master mix by combining only the iScript DNase and iScript DNase Buffer according to the guidelines in Table 2. Mix thoroughly by pipetting up and down several times.

Table 2. Setup for DNase master mix.

Component	Volume per Reaction, µl	Ten Reactions, µl
iScript DNase	0.5	5
iScript DNase Buffer	1.5	15
Total volume	**2**	**20**

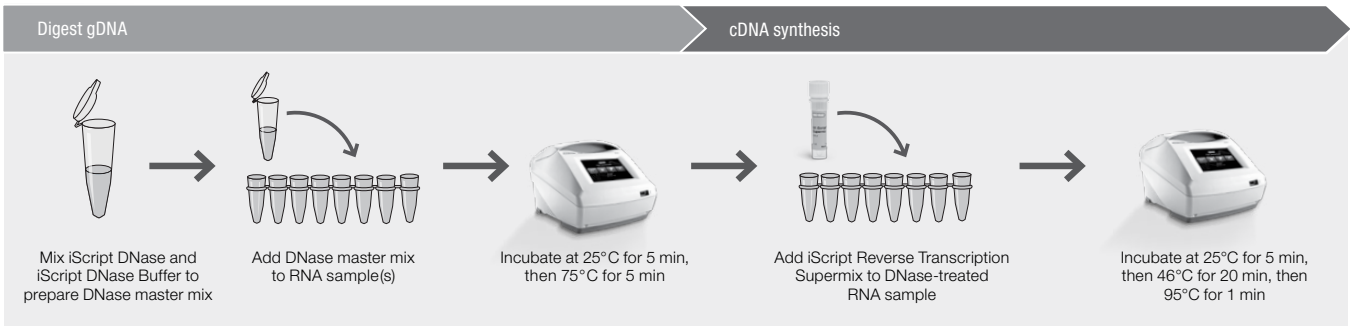

Fig 1. Workflow for reaction setups.

2. Add 2 µl of the DNase master mix to each 14 µl RNA sample or diluted RNA + water sample for a total reaction volume of 16 µl.

ⓘ Input RNA amounts, ranging from 1 µg–1 pg, must be optimized based on target gene abundance and sample availability. Ensuring the quality and purity of the RNA sample is essential for achieving the highest capacity of cDNA.

ⓘ Contamination by gDNA carryover in RNA samples can be tested using the no-RT control supermix. If planning to use the no-RT control, be sure to prepare sufficient DNase-treated RNA for these control cDNA synthesis reactions.

3. Pipet up and down to mix well. If necessary, pulse centrifuge to collect the contents.

4. Using a thermal cycler with the heated lid on, incubate the reaction according to the guidelines in Table 3.

ⓘ For best performance, we recommend using Bio-Rad's C1000™, C1000 Touch™, S1000™, or T100™ Thermal Cycler.

Table 3. DNase reaction protocol.

Step	Temperature, °C	Time, min
DNA digestion	25	5
DNase inactivation	75	5
Storage conditions	4; ice	Until RT step

ⓘ To avoid damage to the RNA, we recommend proceeding directly to cDNA synthesis. However, if longer storage is necessary, store the treated RNA at –80°C or on dry ice and thaw on ice when proceeding with the cDNA synthesis reaction.

Reaction Setup for cDNA Synthesis

1. Make a cDNA synthesis reaction mix by adding the iScript Reverse Transcription Supermix to the DNase-treated RNA template according to the guidelines in Table 4. Mix thoroughly by pipetting up and down several times.

Table 4. Setup for cDNA synthesis reaction.

Component	Volume per Reaction, µl
iScript Reverse Transcription Supermix*	4
DNase-treated RNA template**	16
Total volume	**20**

* For a no-RT control reaction, the iScript Reverse Transcription Supermix should be replaced with iScript No-RT Control Supermix.

** Set up the no-RT control and reverse transcription reactions with the same amount of total RNA. This ensures similar carryover of cDNA synthesis components into subsequent RT-qPCR reactions for accurate detection of gDNA amplicons.

2. Using a thermal cycler with the heated lid on, incubate the complete reaction mix according to the guidelines in Table 5.

Table 5. cDNA synthesis reaction protocol.

Step	Temperature, °C	Time, min
Priming	25	5
Reverse transcription	46	20
RT inactivation	95	1
Hold (optional)	4	—

Recommendations for qPCR

- cDNA generated with this kit can be used directly in qPCR
- The volume of cDNA synthesis reaction used must not exceed 10% of the qPCR volume
- cDNA can be diluted in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA prior to use in qPCR. The optimum cDNA dilution must be determined based on target gene abundance and qPCR chemistry

Related Products

Reverse transcription reagents for real-time qPCR:

- iScript Advanced cDNA Synthesis Kit for RT-qPCR (catalog #172-5038)
- iScript cDNA Synthesis Kit (#170-8891)
- iScript Reverse Transcription Supermix for RT-qPCR (#170-8841)

Reagents for real-time qPCR:

- SsoAdvanced™ Universal SYBR® Green Supermix (#172-5270)
- SsoAdvanced Universal Probes Supermix (#172-5280)
- iTaq™ Universal SYBR® Green Supermix (#172-5120)
- iTaq Universal Probes Supermix (#172-5130)
- SsoAdvanced PreAmp Supermix (#172-5160)

Visit **bio-rad.com/web/gDNAclearkit** for more information.

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