BIO-RAD

# iScript™ RT-qPCR Sample Preparation Reagent

100 x 100 µl preparations (10 ml) 170-8898
500 x 100 µl preparations (5 x 10 ml) 170-8899

For research purposes only.
Store at –20°C.

## Storage and Stability

iScript RT-qPCR sample preparation reagent is stable for 12 months when stored in a constant temperature freezer at –20°C. After thawing, mix thoroughly before using. Repeated freezing and thawing is not recommended. For convenience, it may be stored unfrozen at 2–8°C for up to 6 months.

## Kit Contents

iScript RT-qPCR sample preparation reagent is an optimized buffer designed to deliver efficient cell lysis, RNA stabilization, and removal of genomic DNA for sensitive quantitative gene expression analysis. This novel reagent accelerates and streamlines RT-qPCR analysis of cultured cells by eliminating the need for lengthy RNA purification. Reverse transcription PCR and real-time PCR can be performed directly from cell lysates

| Reagent | Kit Size | Volume | Description |
|---|---|---|---|
| iScript RT-qPCR Sample Preparation Reagent | 100 x 100 µl preps<br>500 x 100 µl preps | 1 x 10 ml<br>5 x 10 ml | Lysis buffer for the isolation and stabilization of cytoplasmic RNA (free of genomic DNA) |

## Quality Control

Functionally, each lot of iScript RT-qPCR sample preparation reagent is tested against predetermined specifications to ensure consistent product quality. iScript RT-qPCR sample preparation reagent is free of contaminating DNases and RNases.

## Instructions for Use

***Note***: *See pilot experiment below to determine the optimal and maximum number of cells that can be processed with this reagent.*

For suspension or adherent cells grown in 6-, 12, or 24-well plates:

1. Seed cells into plate and grow to an optimal density.
2. Aspirate media from plate wells. Wash with an appropriate volume of PBS and aspirate.
3. Add 100 µl of iScript RT-qPCR sample preparation reagent to each well. Incubate for 1 minute at room temperature.
4. Carefully collect the cell lysate without disturbing the cells.
5. Place supernatant on ice for short term storage, –20°C or –80°C for longer term storage.
6. Proceed to the reverse transcription reaction.

For suspension or adherent cells grown in tissue culture flasks:

1. Harvest cells and resuspend in phosphate buffered saline (PBS) to a concentration of 1 x $10^6$ cells/ml.
2. Aliquot a maximum of 1 x $10^5$ cells into a separate microcentrifuge tube. Centrifuge cells at 2000 rcf (relative centrifugal force) at 4°C and remove supernatant. Do not disturb cell pellet, but do not leave more than 5 µl of PBS remaining.
3. Add 100 µl of iScript RT-qPCR sample preparation reagent and vortex on medium setting (eg. setting 6 out of 10) for 30 seconds.
4. Centrifuge lysate at 15,000 rcf for 2 minutes. Carefully collect the supernatant without disturbing the pellet.
5. Place supernatant on ice for short term storage, –20°C or –80°C for longer term storage.
6. Proceed to the reverse transcription reaction.

## Pilot Experiment

To determine the maximum number of a particular cell type that can be used without causing inhibition of RT-qPCR, perform the following experiment:

1. Count cells and resuspend in PBS at a concentration of 5000 cells/µl.
2. Starting with 120 µl of the cell suspension from step 1, perform 5-fold serial dilutions by:
   a. Preparing 5 tubes containing 80 µl PBS on ice.
   b. Transfer 20 µl of original cell suspension (5000 cells/µl) into 80 µl PBS (1:5 dilution or 1000 cells/µl) and mix gently.
   c. Repeat 4 more times to create the following cell suspensions: 5000, 1000, 200, 40, 8, and 1.6 cells/µl.
3. Centrifuge cells at 2000 rcf for 4 min at 4°C and remove supernatant. Do not disturb the cell pellet, but leave no more than 5 µl of PBS remaining.
4. Add 100 µl of iScript RT-qPCR sample preparation reagent and vortex on medium setting (eg. setting 6 out of 10) for 30 sec.
5. Centrifuge lysate at 15,000 rcf for 2 minutes. Carefully collect the supernatant without disturbing the pellet and place on ice.
6. Perform reverse transcription following the instructions provided with Bio-Rad's iScript™ cDNA synthesis kit.
   ***Note***: *The lysate should make up no more than 10% of the final cDNA reaction volume.*
7. Perform qPCR according to the instructions provided with Bio-Rad's iQ™ SYBR® Green supermix or iQ™ supemix.
   a. Choose the appropriate housekeeping gene or gene of interest for your particular cell line (e.g. β-actin, GAPDH, α-tubulin, etc.) and use validated primers (and probes, if applicable).
   ***Note****: The cDNA reaction should make up no more than 10% of the final qPCR reaction volume.*
8. Evaluate results to determine the effective linear range of this reagent with your particular cell type.
   a. Plot Ct values vs. log of the cell concentration to determine the maximum concentration of cells that can be processed by the iScript RT-qPCR sample preparation reagent without inhibiting RT-qPCR.

## Guidelines for Optimal Results Using iScript RT-qPCR Sample Preparation Reagent:

A. After exposing cells to iScript RT-qPCR sample preparation reagent, avoid excessive or rigorous vortexing as this may disrupt the cell nuclei and result in contaminating genomic DNA in the RNA preparation.

B. Use the following table to determine the approximate cell number and RNA amounts in a 2-step RT-qPCR reaction:

| Cells/µl in Lysate | Cells/µl in RT | Cells in 20 µl RT | Cells/µl in PCR | Cells in 20 µl qPCR |
|---|---|---|---|---|
| 1 | 0.1 -- 0.3 | 2 -- 6 | 0.01 -- 0.03 | 0.2 -- 0.6 |
| 10 | 1 -- 3 | 20 -- 60 | 0.1 -- 0.3 | 2 -- 6 |
| 100 | 10 -- 30 | 200 -- 600 | 1 -- 3 | 20 -- 60 |
| 1000 | up to 100 | up to 2000 | up to 10 | up to 200 |

C. Use the following table to determine the approximate cell number and RNA amounts in a 1-step RT-qPCR reaction:

| Cells/µl in Lysate | Cells/µl in RT-qPCR | Cells in 20 µl RT-qPCR |
|---|---|---|
| 1 | 0.1 -- 0.2 | 1 -- 2 |
| 10 | 1 -- 2 | 10 -- 20 |
| 100 | 5 -- 10 | 100 -- 200 |
| 1000 | up to 10 | up to 200 |

D. No more than 10% of the reverse transcription reaction volume should be carried over from the cell lysis step.

E. No more than 10% of the qPCR reaction volume should be carried over from the reverse transcription reaction.

## Reagents and Materials Not Supplied

### cDNA Synthesis kits

iScript cDNA synthesis kit, 170-8891
iScript™ Select cDNA synthesis kit, 170-8897

### Reagents for qPCR

iQ SYBR® Green supermix, 170-8882
iQ supermix, 170-8862
iQ™ multiplex powermix, 172-5849

To learn more about Bio-Rad's complete solution for Amplification, visit our website: **www.bio-rad.com/amplification**

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