TransScript® Mouse Single Cell BCR IgG H/K Amplification Kit

Please read the manual carefully before use

Version No. Version 1.0

Cat. No. AR201

Storage: BCR TSO should be stored at -70℃ or below for one year. All other components should be stored at -18℃ or below for one year.

Description

TransScript® Mouse Single Cell BCR IgG H/K Amplification Kit is specifically designed for the amplification of B-cell receptor (BCR) IgG heavy-chain variable region and kappa light-chain variable region from mouse B cells or total RNA derived from corresponding cells. The kit is compatible with high-throughput workflows, and the amplified products are suitable for downstream applications, including Sanger sequencing, next-generation sequencing (NGS), and gene cloning and expression.

Applications

- 1-10^3 mouse B cells expressing BCR.
- Hybridoma cells (<100 cells per reaction).
- 10 pg-10 ng of total RNA derived from mouse B cells.

Kit Contents

Component	AR201-01 (96 rxns)
BCR Lysis Buffer	96 µl
BCR Oligo(dT)	192 µl
10 mM dNTPs	192 µl
Ribonuclease Inhibitors	96 µl
BCR RT Buffer	480 µl
BCR TSO	96 µl
BCR Reverse Transcriptase	192 µl
2×BCR Amplification SuperMix	2×3.6 ml
BCR cDNA Primer	96 µl
mHeavy Primer	192 µl
mLight Primer	192 µl
RNase-free Water	2×4 ml

Recommended Reagents (Not Supplied)

- *MagicPure*® Size Selection DNA Beads (Cat. No. EC401).
- Pre-chilled 1× PBS solution, freshly prepared 80% ethanol, sterile ultrapure water, etc.

Sample Preparation

- **Cell Sample Preparation**

After collection, cells should be resuspended in 1× PBS to remove culture medium that may interfere with subsequent reactions. Cell viability should be maintained at ≥90%. RNA degradation resulting from low cell viability or impure cellular environment will lead to reduced cDNA yield and smaller fragment size profiles, which can severely compromise library preparation.

• **Single-Cell Sorting**

Single cells should be sorted into reaction wells pre-loaded with the lysis system using methods such as fluorescence-activated cell sorting (FACS). Centrifuge as soon as possible after sorting to ensure that the sorted single cells are fully immersed in the lysis buffer. Since the volumes of both the lysis buffer and the liquid carried by the single cells are very small, immediate centrifugation can prevent cells sorted near the edge of the well from drying on the wall, thereby improving the success rate of library preparation. **The volume of the buffer carried by the sorted single cells is negligible.** The sorted cell samples can be stored at temperatures below -70°C and shipped on dry ice. It is recommended to store them for no more than half a month.

• **RNA Sample Preparation**

Assess RNA integrity before proceeding using methods such as agarose gel electrophoresis or the Agilent RNA 6000 Pico Kit. RNA degradation will result in reduced cDNA yield and smaller fragment size profiles, which can severely compromise library preparation.

Procedures

1.Cell collection and lysis / RNA pretreatment (perform in a clean bench)

(1) For **cell samples**, prepare the following Preserving Buffer (**6 µl**) on ice:

Component	Volume
BCR Lysis Buffer	1 µl
BCR Oligo(dT)	2 µl
10 mM dNTPs	2 µl
Ribonuclease Inhibitors	1 µl
Total volume	**6 µl**

Add the above reaction system to the cell sample, making a total volume of **11 µl**:

Component	Volume
Preserving Buffer	6 µl
Cell Sample	X µl*
RNase-free Water	up to 11 µl
Total volume	**11 µl**

Gently mix the samples, incubate at 72°C for 3 minutes in a thermal cycler, after then immediately place the PCR tube on ice for 2 minutes. Then perform cDNA first-strand synthesis (step 2 in this protocol).

*The volume of the buffer carried by the sorted single cells is negligible. Sort cells directly into 6 µl of Preserving Buffer and adjust the final volume to 11 µl with 5 µl RNase-free Water.

(2) For **RNA samples**, prepare the following Preserving Buffer (**5 µl**) on ice:

Component	Volume
BCR Oligo(dT)	2 µl
10 mM dNTPs	2 µl
Ribonuclease Inhibitors	1 µl
Total volume	**5 µl**

Add the above reaction system to the RNA sample, making a total volume of **11 µl**:

Component	Volume
Preserving Buffer	5 µl
RNA Sample	X µl
RNase-free Water	up to 11 µl
Total volume	**11 µl**

Gently mix the samples, incubate at 72°C for 3 minutes in a thermal cycler, after then immediately place the PCR tube on ice for 2 minutes. Then perform cDNA first-strand synthesis (step 2 in this protocol).

2. cDNA first-strand synthesis (perform in a clean bench)

(1) During ice incubation, prepare the first-strand cDNA synthesis mix (total volume **9 µl**) and place it on ice.

Component	Volume
BCR RT Buffer *	5 µl
BCR TSO	1 µl
BCR Reverse Transcriptase	2 µl
RNase-free Water	1 µl
Total volume	**9 µl**

*BCR RT Buffer should be completely thawed **at room temperature** and **thoroughly vortexed** before use.

(2) Add 9 µl of the first-strand cDNA synthesis mix to the step 1 samples that have completed the 2-minute ice incubation, gently mix with a pipette, and centrifuge briefly, reaching a total volume of **20 µl**.

(3) Run the following program in a thermal cycler (with a heated lid set at 90°C).

Temperature	Time
42°C	90 min
85°C	5 min
4°C	Hold

3. Full-length cDNA amplification (perform in a clean bench)

(1) Add the following components to the samples that have completed the reaction on ice. Reaction system with a total amplification volume of 50 µl:

Component	Volume
The Product of the Previous Step	20 µl
2×BCR Amplification SuperMix	25 µl
BCR cDNA Primer	1 µl
RNase-free Water	4 µl
Total volume	**50 µl**

(3) Vortex the sample thoroughly and centrifuge briefly. Run the following program in a thermal cycler (with a heated lid set at 105°C).

Temperature	Time	Cycle
95°C	3 min	1
98°C 60°C 72°C	20 s 15 s 1 min	20
72°C	5 min	1
4°C	Hold	1

4. Amplification of IgG heavy-chain and light-chain variable regions

(1) Add the following components on ice.

Component	Volume
cDNA Amplification Product	1 µl
mHeavy Primer / mLight Primer*	2 µl
2× BCR Amplification SuperMix	25 µl
RNase-free Water	22 µl
Total volume	**50 µl**

*Select either mHeavy Primer (heavy chain) or mLight Primer (light chain) according to the amplification target.

(2) Mix thoroughly by pipetting and spin briefly.

(3) Run the following program in a thermal cycler.

Temperature	Time	Cycle
95°C	3 min	1
98°C 57°C 72°C	15 s 15 s 30 s	25*
72°C	5 min	1
4°C	Hold	1

*25 cycles are sufficient for H/L chain amplification from typical single B or hybridoma cells. If amplification efficiency is suboptimal, the cycle number may be increased to 30.

(4) Perform quality control or sequencing of the amplified BCR heavy-chain and light-chain products. Fragment lengths (approximately 600-700 bp for both chains) can be verified by agarose gel electrophoresis, Agilent 2100 Bioanalyzer, Qsep, or other suitable methods. The amplified BCR products may also be further analyzed by Sanger sequencing or next-generation sequencing (NGS).

For research use only, not for clinical diagnosis.

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