

SMART-Seq® Mouse BCR (with UMIs)

Catalog Nos.	Amount	Lot Number
634352	24 rxns	Specified on product label.
634353 (Also sold as part of 634351)	96 rxns	Specified on product label.

Description

SMART-Seq Mouse BCR (with UMIs) enables users to analyze mouse B-cell receptor (BCR) diversity from RNA input samples. The kit is designed to work with a range of RNA input amounts depending on the sample type and has been shown to generate high-quality libraries from as little as 10 ng–1 µg of total RNA obtained from spleen, bone marrow, whole blood, and peripheral blood mononuclear cells (PBMCs).

The kit leverages SMART® (Switching Mechanism at 5' end of RNA Template) technology and employs a 5' RACE-like approach to capture complete V(D)J variable regions of BCR transcripts. Included in the kit are primers that incorporate Illumina®-specific adaptor sequences during cDNA amplification. The workflow incorporates unique molecular identifiers (UMIs), providing greater accuracy for clonotype identification and quantification during data analysis. Using the Unique Dual Index kits (Cat. Nos. 634752–634756, sold separately), the protocol can generate up to 384 multiplexed libraries that are ready for sequencing on any Illumina platform.

Package Contents

Package 1:

<u>634352</u> (24 rxns)	<u>634353</u> (96 rxns)	
5 µl	5 µl	Control RNA (1 µg/µl)
24 µl	96 µl	SMART UMI Oligo

Package 2:

<u>634352</u> (24 rxns)	<u>634353</u> (96 rxns)	
48 µl	192 µl	dT Primer
96 µl	384 µl	First-Strand Buffer (5X)
48 µl	192 µl	SMARTScribe™ Reverse Transcriptase (100 U/µl)
5 ml	20 ml	Nuclease-Free Water
24 µl	96 µl	RNase Inhibitor (40 U/µl)
24 µl	96 µl	BCR Enhancer
1 ml	4 x 1 ml	Elution Buffer (10 mM Tris HCl, pH 8.5)
24 µl	96 µl	mBCR PCR1 Universal Forward
24 µl	96 µl	mBCR PCR1 Reverse
24 µl	96 µl	mBCR PCR2 HC Reverse
24 µl	96 µl	mBCR PCR2 LC Reverse
2 x 1 ml	8 x 1 ml	PrimeSTAR® GXL Premix (2X)

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Certificate of Analysis

SMART-Seq Mouse BCR (with UMIs)

Cat. Nos. 634352 & 634353

Also sold as part of 634351

Storage Conditions

- Store Control RNA and SMART UMI Oligo at -70°C .
- Store BCR Enhancer and Nuclease-Free Water at -20°C . Once thawed, they can be stored at 4°C .
- Store Elution Buffer at -20°C . Once thawed, the buffer can be stored at room temperature.
- Store all other reagents at -20°C .

Expiration Date

- Specified on product label.

Shipping Conditions

- Dry ice

Product Documents

Documents for our products are available for download at takarabio.com/manuals

The following documents apply to this product:

- SMART-Seq Mouse BCR (with UMIs) User Manual
- Unique Dual Index Kits Protocol-At-A-Glance

Quality Control Data

A sample kit from each lot was tested as follows: 10 ng of Control RNA (Mouse Spleen Total RNA) was subjected to first-strand cDNA synthesis as described in the SMART-Seq Mouse BCR (with UMIs) User Manual. The first-strand cDNA was then used as a template for all seven isotypes (IgA/D/E/G/M/K/L) in a PCR reaction, each for 16 cycles of PCR (PCR 1). The heavy chains (IgA/D/E/G/M) and the light chains (IgK/L) were amplified separately in the second PCR (PCR 2). 1 μl of the resulting double-stranded cDNA was amplified by nested PCR for 20 cycles for corresponding heavy chains and light chains. The final sequencing libraries were purified using NucleoMag NGS Clean-up and Size Select (Takara Bio; Cat. Nos. 744970.5, 744970.50, or 744970.500) and resuspended in 17 μl of Elution Buffer.

2 μl of each sequencing library was quantified by Qubit Fluorometer and Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat No. Q32851 or Q32854), and 1 μl of each sequencing library was analyzed with an Agilent 2100 Bioanalyzer and the DNA High Sensitivity Kit (Agilent Technology, Cat. No. 5067-4626). Qubit assay indicated the yield of both heavy-chain library and light-chain library was ≥ 3 ng/ μl . The following Bioanalyzer analysis indicated that all library profiles produced a well-defined peak with size between 600–900 bp region on an electropherogram. Size distributions as below:

- Heavy-chain library: 680 bp (+/- 60 bp)
- Light-chain library: 640 bp (+/- 60 bp)

NOTE: Occasionally, a high molecular peak might be observed in the electropherogram around 2,000–10,000 bp, with an average size of approximately 4,500 bp, which is possibly due to overamplification. This high molecular weight peak does not affect the sequencing and downstream data.

All primers are verified by ESI analysis to have a major peak with a calculated molecular weight within 30 Da of the predicted molecular weight.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

SMART-Seq® Mouse BCR (with UMIs)

CATALOG NOS.

634352 & 634353

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

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STATEMENT 392

This Product is protected by one or more patents from the family consisting of: US10941397, People's Republic of China Patent: ZL201480057094.4, US10781443, US10954510, DE602014069266.4, EP3058104, FR3058104, UK3058104, JP6602294, SE3058104, CA2923812, and any corresponding patents, divisionals, continuations, patent applications and foreign filings sharing common priority with the same family. Information on relevant patents and licenses for this product may be found at: <https://www.takarabio.com/patents>.

STATEMENT 455

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