

SMARTer® Human BCR IgG IgM H/K/L Profiling Kit

Catalog Nos.	Amount	Lot Number
634466	12 rxns	Specified on product label.
634467	48 rxns	Specified on product label.

Description

The SMARTer Human IgG IgM H/K/L BCR Profiling Kit enables users to analyze human 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 to 1 µg of total RNA from peripheral blood mononuclear cells (PBMCs) and 1 to 100 ng of total RNA from B cells.

The kit leverages SMART® 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 protocol generates indexed libraries that are ready for sequencing on Illumina platforms.

Package Contents

Package 1:

<u>634466</u> (12 rxns)	<u>634467</u> (48 rxns)	
5 µl	5 µl	Control RNA (1 µg/µl)
15 µl	60 µl	SMART UMI Oligo

Package 2:

<u>634466</u> (12 rxns)	<u>634467</u> (48 rxns)	
300 µl	1 ml	BCR Enhancer
60 µl	230 µl	First-Strand Buffer (5X)
30 µl	120 µl	dT Primer
30 µl	120 µl	SMARTScribe™ Reverse Transcriptase (100 U/µl)
30 µl	120 µl	RNase Inhibitor (40 U/µl)
1.7 ml	3 x 1.7 ml	Elution Buffer (10 mM)
5 ml	20 ml	Nuclease-Free Water
200 µl	600 µl	PrimeSTAR® GXL DNA Polymerase (1.25 U/µl)
2 x 1 ml	6 ml	5X PrimeSTAR GXL Buffer
800 µl	2.4 ml	dNTP Mixture (2.5 mM each)
60 µl	225 µl	hBCR PCR1 Universal Forward
15 µl	60 µl	hBCR PCR1 IgG Reverse
15 µl	60 µl	hBCR PCR1 IgM Reverse
15 µl	60 µl	hBCR PCR1 IgK Reverse
15 µl	60 µl	hBCR PCR1 IgL Reverse

Takara Bio USA, Inc.

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Certificate of Analysis

Cat. Nos. 634466, 634467

SMARTer Human BCR IgG IgM H/K/L Profiling Kit

Package 3:

<u>634466</u> (12 rxns)	<u>634467</u> (48 rxns)	
15 µl	15 µl	hBCR PCR2 IgG Reverse 1
–	15 µl	hBCR PCR2 IgG Reverse 2
–	15 µl	hBCR PCR2 IgG Reverse 3
–	15 µl	hBCR PCR2 IgG Reverse 4
15 µl	15 µl	hBCR PCR2 IgM Reverse 1
–	15 µl	hBCR PCR2 IgM Reverse 2
–	15 µl	hBCR PCR2 IgM Reverse 3
–	15 µl	hBCR PCR2 IgM Reverse 4
15 µl	15 µl	hBCR PCR2 IgK Reverse 1
–	15 µl	hBCR PCR2 IgK Reverse 2
–	15 µl	hBCR PCR2 IgK Reverse 3
–	15 µl	hBCR PCR2 IgK Reverse 4
15 µl	15 µl	hBCR PCR2 IgL Reverse 1
–	15 µl	hBCR PCR2 IgL Reverse 2
–	15 µl	hBCR PCR2 IgL Reverse 3
–	15 µl	hBCR PCR2 IgL Reverse 4
15 µl	60 µl	hBCR PCR2 Forward 1
15 µl	60 µl	hBCR PCR2 Forward 2
15 µl	60 µl	hBCR PCR2 Forward 3
15 µl	60 µl	hBCR PCR2 Forward 4
15 µl	60 µl	hBCR PCR2 Forward 5
15 µl	60 µl	hBCR PCR2 Forward 6
15 µl	60 µl	hBCR PCR2 Forward 7
15 µl	60 µl	hBCR PCR2 Forward 8
15 µl	60 µl	hBCR PCR2 Forward 9
15 µl	60 µl	hBCR PCR2 Forward 10
15 µl	60 µl	hBCR PCR2 Forward 11
15 µl	60 µl	hBCR PCR2 Forward 12

Storage Conditions

- Store Control RNA and SMART UMI Oligo at –70°C.
- Store BCR Enhancer at –20°C. Once thawed, the buffer can be stored at 4°C.
- Store Nuclease-Free Water at –20°C. Once thawed, the water 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.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

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Shipping Conditions

- Dry ice (–70°C)

Product Documents

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

The following documents apply to this product:

- SMARTer Human BCR IgG IgM H/K/L Profiling Kit User Manual

Quality Control Data

A sample kit from each lot was tested as follows: 10 ng of Control RNA (Human Spleen Total RNA) was subjected to first-strand cDNA synthesis as described in the SMARTer Human BCR IgG IgM H/K/L Profiling Kit User Manual. The first-strand cDNA was then used as a template in four different PCRs for heavy chains and kappa and lambda light chains, each for 18 cycles of PCR. 1 µl of the resulting double-stranded cDNA was amplified by nested PCR for 20 cycles for corresponding heavy chains and kappa and lambda light chains. The final sequencing library was purified with an Agencourt AMPure XP Kit (Beckman Coulter, Part No. A63880 or A63881) and resuspended in 17 µl of Elution Buffer. 2 µl of the sequencing library was analyzed by Qubit Fluorometer and Qubit dsDNA HS Assay Kit (Cat. No. Q32854), and then 1 µl of the sequencing library was analyzed with an Agilent 2100 Bioanalyzer and a DNA 1000 Kit (Agilent, Cat. No. 5067-1504). The Qubit assay indicated the yields of IgG heavy chain libraries are more than 10 ng/µl, IgM heavy chain libraries are more than 7 ng/µl, and kappa (IgK) and lambda (IgL) light chain libraries are more than 10 ng/µl. The subsequent Bioanalyzer analysis indicated that all the library profiles produced a single peak with its size between 600–900 bp on an electropherogram image. Size distributions were as listed below:

IgG library: 730 bp (+/– 60 bp)

IgM library: 750 bp (+/– 60 bp)

IgK library: 630 bp (+/– 60 bp)

IgL library: 650 bp (+/– 60 bp)

All PCR primers were PAGE-purified and verified by ESI analysis.

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

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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.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, and described on the product's web page at <http://www.takarabio.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

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Patent pending. For further information, please contact a Takara Bio USA licensing representative by email at licensing@takarabio.com.

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6/24/2019