Manual

Version 5.0

Product name: Ab Cloning 5' RACE Kit

Cat #: ABC5-100, ABC5-200, ABC5-OEM

Description:

The Ab Cloning 5' RACE Kit provides a novel method for performing 5′ rapid amplification of cDNA ends (RACE) with optimized primers for cloning both of heavy and light chain of human and mouse antibodies. MCLAB's SmartRT Reverse Transcriptase is an engineered MMLV RT that improves the enzyme's thermostability, cDNA synthesis ability, and reduces RNase H activity. The enzyme also has a terminal transferase activity. The tailing activity (terminal transferase activity) of SmartRT Reverse Transcriptase allows you to synthesize complete cDNA by SMART (Switching Mechanism At 5′ end of RNA Transcript) cDNA synthesis technology. With 3-5 extra nuclear acids added to the 3′ end of the first-strand cDNA, the 5′ SMART Universal Oligo contains a terminal complementation to the nuclear acids at 3′ end of the first-strand cDNA, and can be annealing to first-strand cDNA tail and serves as an extended template for RT. The switch of templates from mRNA to 5′ SMART Universal Oligo produces a complete cDNA copy of transcript RNA with 5′ SMART Universal Oligo at the end. The cDNA transcript from smartRT can be used directly in 5′ RACE with MCLAB I-5™ 2X High-Fidelity Master Mix. Our Ab Cloning 5′ Race Kit is optimized for antibody cloning. It also can be used for any gene of your interest.

Features:

- Specific full length cDNA enrichment technique
- Optimized primer set for full length antibody sequencing
- Simplified protocol without 5′ end adaptor-ligation step

Components:

Cat#: ABC5-100 Ab Cloning 5′ RACE Kit (10 reactions)

1.

Components	Cap Color	Volume (10 rxn)	Volume (20 rxn)
Oligo dT Primer	Orange	10 µl	20 µl
5X RT Reaction Buffer	Green	40 µl	80 µl
dNTP	Yellow	10 µl	20 µl
DTT	Yellow	5 µl	10 µl
SmartRT™ Reverse Transcriptase	Clear	20 µl	40 µl
5′ PCR Universal Primer	Red	10 µl	20 µl
3′ IgG Primer	Red	10 µl	20 µl
3′ IgM Primer	Red	10 µl	20 µl
3′ IgE Primer	Red	10 µl	20 µl
3′ Kappa Chain Primer	Red	10 µl	20 µl
3′ Lambda Chain Primer	Red	10 µl	20 µl
I-5™ 2X High-Fidelity Master Mix	Purple	100 µl	200 µl
RNAse Inhibitor	Blue	5 µl	10 µl

Storage Condition: -20 °C

2. 5′ SMART Universal Oligo, 10 µl/20 µl. Storage Condition: -80 °C

Protocol:

For preparation of 5'-RACE-Ready cDNA

Combine the followings in separate 0.2-ml PCR tubes (20 µl reaction):

2 µg	RNA sample
1 µl	Oligo dT Primer

Add DEPC H_2O to a final volume of 11 µl for each reaction. Incubate the tubes at: 72°C for 3 min
42°C for 2 min
4°C hold

Add the followings to each reaction tube:

4 µl	5X First-Strand Buffer
0.5 µl	DTT (100 mM)
1 µl	dNTP Mix (10 mM)
1 µl	5′ SMART Universal Oligo
0.5 µl	RNase Inhibitor (40U/µl)
2 µl	SmartRT Reverse Transcriptase (100U/µl)

Incubate the tubes at: 42°C for 1.5 hr
70°C for 10 min
4°C hold

* You can dilute the first-strand cDNA synthesis reaction product with Tricine-EDTA Buffer if your gene of interest is abundant.
* The 5′-RACE-Ready cDNA samples can be stored at –20°C for up to three months.

PCR Protocol

10 µl	I-5™ 2X High-Fidelity Master Mix	
1 µl	5′ PCR Universal Primer (10µM)	
1 µl	3′ Primer	For heavy chain (IgG 3′ primer, IgM 3′ primer or IgE 3′ primer) For light chain (kappa chain primer or lambda chain primer)
2 µl	cDNA template	
6 µl	sterile water	

PCR Program

Touch down PCR (recommended)

94°C 30 sec, 72°C 3 min	5 cycles
94°C 30 sec, 70°C 30 sec, 72°C 3 min	5 cycles
94°C 30 sec, 68°C 30 sec, 72°C 3 min	20-25 cycles

4°C Hold

* If fragments >3 kb are expected, add 1 minute for each additional 1 kb.
* 68°C can be adjusted at a range from 60-68°C for the best results.
* The PCR product can be detected on a 1% agarose gel with the size range from 500 to 700bp.
* Multiple bands may be observed due to the purity of the starting material. Agarose gel purification is highly recommended to eliminate the undesired PCR bands.

Detailed Flow Chart of Ab 5' RACE