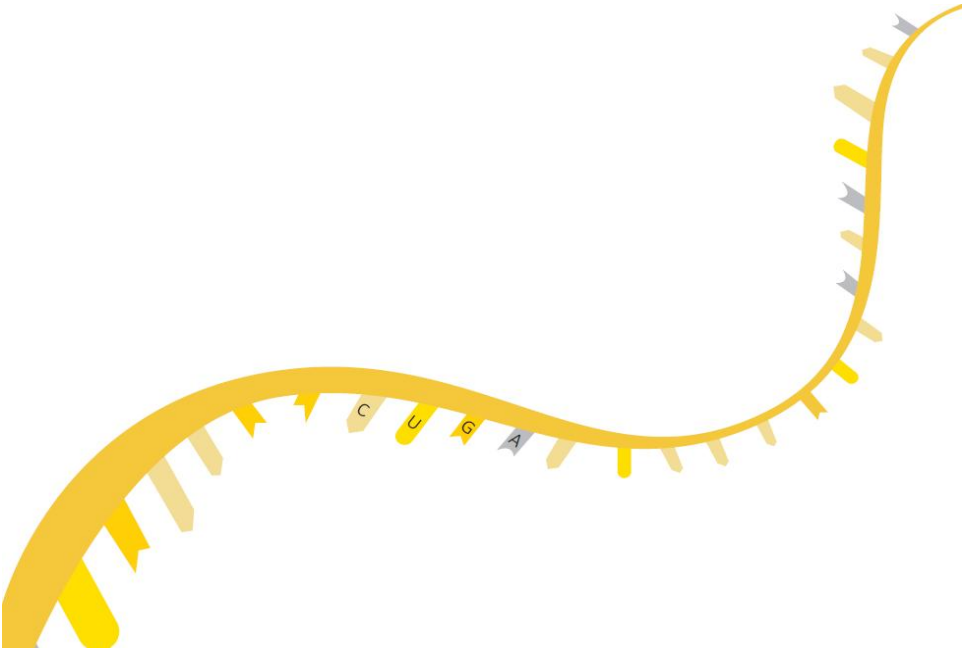
# ABclonal Second Strand Synthesis Module

## RK20346

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version: N14C16v1.1

# Contents

# 1. Introduction

The ABclonal Second Strand Synthesis Module (RK20346) uses product from the ABclonal First Strand cDNA Synthesis Module (RK20342 or RK20353) as a template for second strand cDNA synthesis to generate a double-stranded cDNA product. This double-stranded cDNA product can be directly used for DNA library construction.

# 2. List of Components

**Table 1. Kit Contents**

| | Tube name | 24RXN | 96RXN | 500RXN |
|---|---|---|---|---|
| ● | Second Strand Synthesis Reaction Buffer | 192 µL | 768 µL | 4 mL |
| ● | Second strand Synthesis Enzyme Mix | 96 µL | 384 µL | 1 mL X 2 |
| ○ | Nuclease-free Water | 2 mL | 8 mL | - |

# 3. Storage

All components should be stored at - 20℃.

# 4. Additional Materials Required

First Strand Synthesis cDNA: ABclonal First Strand cDNA Synthesis Module (RK20342 or RK20353).

Purification Beads: AFTMag NGS DNA Clean Beads (ABclonal, cat. no. RK20257).

Other Materials: Nuclease-Free Water, 100% ethanol, Vortex mixer, Low absorption EP tubes, PCR tubes, magnetic stand, PCR instrument.

# 5. Protocol

## Step 1. RNA First Strand Synthesis

The ABclonal First Strand Synthesis Module (RK20342 or RK20353) is recommended to prepare first strand cDNA products.

## Step 2. Second Strand Synthesis

2.1 Thaw the Second Strand Synthesis Reaction Buffer on ice, then add the reagents to a PCR tube in sequence according to the following table:

**Table 2. Second Strand Synthesis Reaction Setup (per sample)**

| | Component | Volume |
|---|---|---|
| | First Strand cDNA | 20 μL |
| ● | Second Strand Synthesis Reaction Buffer | 8 μL |
| ● | Second Strand Synthesis Enzyme Mix | 4 μL |
| ○ | Nuclease-free Water | 48 μL |
| | **Total volume** | **80 μL** |

2.2 Use a pipette to mix evenly, then centrifuge briefly and place the sample in a PCR machine (with the heated lid closed), and incubate at 16°C for 1 hr.

## Step 3. Purification with Magnetic Beads

3.1 The AFTMag NGS DNA Clean Beads should be equilibrated to room temperature for 30 min prior to use to maximize recovery efficiency and sorting accuracy.

3.2 Add 144 µL (1.8X) of AFTMag NGS DNA Clean Beads to each sample, mix well, and incubate at room temperature for 5 min.

3.3 Place the PCR tube on a magnetic stand and allow it to stand for 2 min, then remove the supernatant after the solution becomes clear (take care not to touch the magnetic beads).

3.4 With the PCR tube on the magnetic stand, gently rinse the beads by adding 200 µL of freshly prepared 80% ethanol to the tube. Incubate the beads and sample for 30 sec on the magnetic stand, then carefully remove the supernatant.

3.5 Repeat Step 3.4.

3.6 With the PCR tube on the magnetic stand, remove any residual ethanol from the bottom of the tube using a 10 µL pipette, then open the cap and allow the tube to dry until there is no remaining ethanol residue.

3.7 Remove the PCR tube from the magnetic stand, add 39 µL of Nuclease-Free Water to resuspend the magnetic beads, and allow the beads to stand at room temperature for 1 min to fully release the DNA from the beads.

3.8 Return the PCR tube to the magnetic stand and allow it to stand for 2 min. Once the solution is clear of beads, transfer 37 µL of the supernatant to a new PCR tube for storage until analysis.

3.9 The purified DNA fragmentation products can be stored at -20°C or

can be directly ligated to a conventional DNA library construction kit for further experiments.

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