C&E ExpressArt
Micro dsDNA Synthesis Add-On Module for Combination with mRNA Amplification kits

Bacterial Micro kit Cat.-No. 5199-A30
TR Micro kit Cat.-No. 6199-A30

Catalogue No. 8224-A30

(30 reactions: Conversion of amplified RNA to dsDNA)

Content Kit box I (C&E Micro dsDNA Add-On Module)

Tube 1:	**EMPTY: not required**	**---**
Tube 2:	**dNTP-Mix**	**150.0 µl**
Tube 3:	**DEPC-H_2O**	**1500 µl**
Tube 4:	**5x RT Buffer**	**300.0 µl**
Tube 5:	**RNase Inhibitor**	**24.0 µl**
Tube 6:	**RT Enzyme**	**60.0 µl**
Tube 7:	**RNase**	**30.0 µl**
Tube 8:	**Primer BS (Special)**	**75.0 µl**
Tube 9:	**5x Extender Buffer**	**390.0 µl**
Tube 10:	**EMPTY: not required**	**---**
Tube 11:	**Primer Erase**	**60.0 µl**
Tube 12:	**Primer CS (Special)**	**375.0 µl**
Tube 13:	**Extender Enzyme B**	**75.0 µl**
Tubes 14-20:	**EMPTY: not required**	**---**

Content Kit box II

cDNA Purification Spin Columns	**30 pcs**
Collection Tubes	**30 pcs**
Binding Buffer	**7.5 ml**
Washing Buffer (salt concentrate)	**4 ml**
Elution Buffer	**10 ml**

Storage

Immediately upon arrival:
Store **Kit box I at -20°C**. Avoid repeated freeze thawing.
Kit box II and box III are stored at room temperature.
Reagents are stable for a minimum of 6 months.

Additionally required materials

- Thermocycler. All reactions, apart from the overnight *in vitro* transcription (see below) can be performed in a standard thermocycler (with the lid temperature adjusted to 110℃).
- RNase-free pipette-tips (filter-tips recommended)
- RNase-free PCR and reaction tubes (0.2 / 0.5 / 1.5 ml)
- 100% Ethanol
- Microcentrifuge

Chemical hazards

The Binding Buffer (**box II**) contains guanidine thiocyanate, which is harmful in contact with skin, when inhaled or swallowed. Guanidine thiocyanate also liberates toxic gas, when mixed with strong acids. Always store and use the Binding Buffer away from food. Always wear gloves, and follow standard safety precautions during handling and make sure to comply with the safety rules of your laboratory.

Quality control

All components of the kit are tested in a reaction using amplified RNA obtained from the Positive Control RNA (**Tube 17**, provided with ExpressArt mRNA amplification kits). All reagents are tested for the absence of nuclease activity.

DETAILED PROTOCOL

Conversion of aRNA to dsDNA

DS 1. First strand cDNA synthesis

RNA yields should be determined by photometric **and** electrophoretic measurements (see step A6).
We recommend to use **5 – 10 µg amplified RNA** (a maximum volume of 25 µl of aRNA, obtained with ExpressArt Micro mRNA amplification kits: step A5).

First Strand cDNA Synthesis Mix 2-1		
dNTP-Mix	Tube 2	2.5 µl
Primer BS (special)	Tube 8	2.5 µl

Add 5 µl **Mix 2-1** to 25 µl of each RNA (for smaller volumes, adjust with water to a reaction volume of 30 µl).
Incubate 4 minutes at 65℃ in a thermocycler **(with heating lid! use standard setting, e.g. 110℃)**

Cool samples to 37℃.

In the meantime, prepare the First Strand cDNA Synthesis **Mix 2-2** at room temperature.

First Strand cDNA Synthesis Mix 2-2		
DEPC-H_2O	Tube 3	7.2 µl
5x RT Buffer	Tube 4	10.0 µl
RNase Inhibitor	Tube 5	0.8 µl
RT Enzyme	Tube 6	2.0 µl

Add the First Strand cDNA Synthesis **Mix 2-2** (20 µl) to each sample and mix well by gently flicking the tube.

Incubate the samples in a thermocycler:

37℃ / 45 min
45℃ / 15 min
50℃ / 5 min
37℃ / HOLD

Primer Erase Mix 2-3		
DEPC-H_2O	Tube 3	2 µl
5x Extender Buffer	Tube 9	1 µl
Primer Erase	Tube 11	2 µl

Then add 5 µl Primer Erase **Mix 2-3**, and continue incubations:
37℃ / 5 min
80℃ / 15 min
37℃ / HOLD

RNase Mix 2-4		
DEPC-H_2O	Tube 3	3 µl
5x Extender Buffer	Tube 9	1 µl
RNase	Tube 7	1 µl

Add 5 µl of RNase **Mix 2-4** to First Strand cDNA Reaction.

Incubate 20 minutes at 37℃.

DS 2. dsDNA synthesis

Second Strand cDNA Synthesis Mix 2-5		
DEPC-H_2O	Tube 3	10.0 µl
5x Extender Buffer	Tube 9	10.0 µl
Primer CS (Special)	Tube 12	12.5 µl
dNTP-Mix	Tube 2	2.5 µl

Add 35 µl of **Mix 2-5** to each First Strand cDNA Synthesis Reaction and incubate as follows in a thermocycler:

96℃ / 1 min
37℃ / 1 min

Extender Enzyme B Mix 2-6		
DEPC-H_2O	Tube 3	16.5 µl
5x Extender Buffer	Tube 9	1 µl
Extender Enzyme B	Tube 13	2.5 µl

Add 20 µl of Extender Enzyme B **Mix 2-6** to each sample and mix well by gently flicking the tube. Continue the incubation:

37℃ / 30 min
65℃ / 15 min
4℃ / HOLD

After incubation, place tubes on ice. **Proceed to step DS 3.**

DS 3. Purification of dsDNA with Spin Columns

Before starting, add 16 ml of 100% ethanol to the 4 ml Washing Buffer concentrate (Kit box II) and mix well.

Purification Mix 2-7		
Binding Buffer	(box II)	234 µl
~~no Carrier DNA,~~ instead add H_2O	Tube 3	2 µl

- Add 236 µl of **Mix 2-7** to each **Template DNA Reaction** (115 µl from step DS 2). Mix gently by pipetting.
  Note: The addition of Carrier DNA could interfere with further processing for sequencing.
- Insert DNA Purification Spin Columns in Collection Tubes.
- Pipette the **entire sample** onto each column and centrifuge for 1 min at 10,000 rpm in a table top centrifuge. (**Note:** guanidine thiocyanate in the **Binding Buffer** is an irritant. Always wear gloves and follow standard safety precautions to minimise contact when handling).
- Discard the flow-through and re-insert the columns in the same Collection Tubes. Add 200 µl **Washing Buffer** (with Ethanol added) to the columns and centrifuge for 1 min at 10,000 rpm.
- Discard the flow-through, re-insert the columns in the same Collection Tubes and wash again with 200 µl **Washing Buffer**. Centrifuge for 1 min at 10,000 rpm.
- Discard the flow-through, re-insert the columns in the same Collection Tubes and centrifuge for 1 min at 10,000 rpm. Discard the flow-through and the Collection Tubes.
- Insert the columns in fresh 1.5 ml reaction tubes and add 10 µl of **Elution Buffer** to the columns (make sure to pipette the Elution Buffer exactly in the middle of the column, directly on top of the matrix, without disturbing the matrix with the pipette tip). Incubate the column for at least 2 min, then centrifuge for 1 min at 10,000 rpm.
- The purified dsDNA (approximately 8 µl) is now ready for downstream applications, e.g. PCR amplification.
- Or, store the samples at –20°C for later use.

Thermocycler profiles

Before starting the kit protocol, program a thermocycler with the following temperatures and times. HOLD steps are included to provide time for thermal ramping or for adding reagents.

Generation of dsDNA

Thermocycler program for steps DS1-DS2			
Step	**Temperature**	**Time**	**Action**
1	**65℃**	HOLD	Start of first cDNA synthesis Add **25µl RNA-a1** to **5µl Mix 2-1**
2	**65℃**	**4 min**	
3	**37℃**	**1 min**	
4	**37℃**	HOLD	add **20µl Mix 2-2**
5	**37℃**	**45 min**	
6	**45℃**	**15 min**	
7	**50℃**	**5 min**	
8	**37℃**	**1 min**	
9	**37℃**	HOLD	add **5µl Mix 2-3**
10	**37℃**	**5 min**	
11	**80℃**	**15 min**	
12	**37℃**	**1 min**	
13	**37℃**	HOLD	add **5µl Mix 2-4**
14	**37℃**	**20 min**	
15	**37℃**	HOLD	add **35µl Mix 2-5**
16	**96℃**	**1 min**	
17	**37℃**	**1 min**	
18	**37℃**	HOLD	add **20µl Mix 2-6**
19	**37℃**	**30 min**	
20	**65℃**	**15 min**	
21	**4℃**	HOLD	
End of dsDNA synthesis / **Spin** to collect liquid **Continue** with DNA purification			

Required time: appr. 2.8 h

AmpTec GmbH
Koenigstrasse 4a | 22767 Hamburg | Germany
Tel: +49 (0)40 – 636 747 22 | Fax: +49 (0)40 – 636 747 19

Technical Support:
"Dr. Guido Krupp" <krupp@amp-tec.com>
"Dr. Peter Scheinert" < scheinert@amp-tec.com >
www.amp-tec.com