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

## Bacterial Nano kit Cat.-No. 5299-A15
## TR Nano kit Cat.-No. 6299-A15

## Catalogue No. 8225-A15
## (15 reactions: Conversion of amplified RNA to dsDNA)

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

| | | |
|---|---|---|
| **Tube 1:** | **EMPTY: not required** | **---** |
| **Tube 2:** | **dNTP-Mix** | **75.0 µl** |
| **Tube 3:** | **DEPC-$H_2O$** | **1500 µl** |
| **Tube 4:** | **5x RT Buffer** | **150.0 µl** |
| **Tube 5:** | **RNase Inhibitor** | **12.0 µl** |
| **Tube 6:** | **RT Enzyme** | **30.0 µl** |
| **Tube 7:** | **RNase** | **15.0 µl** |
| **Tube 8:** | **EMPTY: not required** | **---** |
| **Tube 9:** | **5x Extender Buffer** | **165.0 µl** |
| **Tube 10:** | **EMPTY: not required** | **---** |
| **Tube 11:** | **EMPTY: not required** | **---** |
| **Tube 12:** | **Primer CS (Special)** | **180.0 µl** |
| **Tube 13:** | **Extender Enzyme B** | **37.5 µl** |
| **Tube 14:** | **EMPTY: not required** | **---** |
| **Tube 15:** | **Primer DS (Special)** | **75.0 µl** |
| **Tube 16:** | **Reaction Additive** | **75.0 µl** |
| **Tubes 17-24:** | **EMPTY: not required** | **---** |

## Content Kit box II

| | |
|---|---|
| **cDNA Purification Spin Columns** | **15 pcs** |
| **Collection Tubes** | **15 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 17.5 µl of aRNA, obtained with ExpressArt TR Nano mRNA amplification kits: step B6).

| First Strand Mix 3-1 | | |
|---|---|---|
| dNTP-Mix | Tube 2 | 2.5 µl |
| Primer DS (special) | Tube 15 | 5.0 µl |
| Reaction Additive | Tube 16 | 5.0 µl |

Add 12.5 µl of **Mix 3-1** to 17.5 µl RNA (for smaller volumes, adjust with water to a total volume of 30 µl).
Incubate 4 min at 65℃ in a thermocycler (with heating lid! use standard temperature setting, e.g. 110℃), then immediately cool the samples to 45℃.

In the meantime, prepare the First Strand cDNA Synthesis **Mix 3-2**, at room temperature.

| cDNA Synthesis Mix 3-2 | | |
|---|---|---|
| DEPC-$H_2O$ | Tube 3 | 8.4 µl |
| 5x RT Buffer | Tube 4 | 10.0 µl |
| RNase Inhibitor | Tube 5 | 0.8 µl |
| RT Enzyme | Tube 6 | 2.0 µl |

Add 20 µl of **Mix 3-2** to each sample. **Remove one sample at a time** with continued incubation of the other samples in the 45ºC hot thermocycler.

Continue incubation in a thermocycler:

45℃ / 30 min
70℃ / 15 min

## DS 2. RNA removal

| RNase Mix 3-3 | | |
|---|---|---|
| DEPC-$H_2O$ | Tube 3 | 3 µl |
| 5x Extender Buffer | Tube 9 | 1 µl |
| RNase | Tube 7 | 1 µl |

Add 4 µl of RNase **Mix 3-3** to 50 µl of First Strand cDNA Reaction (from C1).
Incubate 20 minutes at 37℃.

## DS 3. dsDNA synthesis

| Second Strand cDNA Synthesis Mix 3-4 | | |
|---|---|---|
| DEPC-$H_2O$ | Tube 3 | 25.5 µl |
| Primer CS (Special) | Tube 12 | 12.0 µl |
| 5x Extender Buffer | Tube 9 | 10.0 µl |
| dNTP-Mix | Tube 2 | 2.5 µl |

Add 50 µl of **Mix 3-4** to each sample (from C2), then incubate:

96℃ / 1 min
37℃ / 1 min

| Extender Enzyme B Mix 3-5 | | |
|---|---|---|
| DEPC-$H_2O$ | Tube 3 | 1.5 µl |
| 5x Extender Buffer | Tube 9 | 1 µl |
| Extender Enzyme B | Tube 13 | 2.5 µl |

Add 5 µl of Extender Enzyme B **Mix 3-5** to each sample and mix well by gently flicking the tube. Continue the incubation:

37℃ / 30 min
65℃ / 15 min

Place the samples on ice. Spin briefly to collect liquid. Continue immediately with purification of template DNA (step DS 4).

## DS 4. 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) | 224 µl |
| ~~no Carrier DNA,~~ instead add $H_2O$ | Tube 3 | 2 µl |

- Add 226 µl of **Mix 2-7** to each **Template DNA Reaction** (110 µl from step DS 3). 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℃ 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-DS3**<br>***For 3rd Amplification Round*** | | | |
|---|---|---|---|
| **Step** | **Temperature** | **Time** | **Action** |
| **1** | **65℃** | HOLD | Start of first cDNA synthesis<br>Add **17.5µl RNA-a1** to **12.5µl Mix 3-1** |
| **2** | **65℃** | **4 min** | |
| **3** | **45℃** | **1 min** | |
| **4** | **45℃** | HOLD | add **20µl Mix 3-2** |
| **5** | **45℃** | **30 min** | |
| **6** | **70℃** | **15 min** | |
| **7** | **37℃** | **1 min** | |
| **8** | **37℃** | HOLD | add **5µl Mix 3-3** |
| **9** | **37℃** | **20 min** | |
| **10** | **37℃** | HOLD | add 3**5µl Mix 3-4** |
| **11** | **96℃** | **1 min** | |
| **12** | **37℃** | **1 min** | |
| **13** | **37℃** | HOLD | add **20µl Mix 3-5** |
| **14** | **37℃** | **30 min** | |
| **15** | **65℃** | **15 min** | |
| **16** | **4℃** | HOLD | |
| **End** of Template DNA synthesis<br>**Spin** to collect liquid<br>**Continue** with DNA purification | | | |

**Required time: appr. 2.2 h**

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