ExpressArt® TR cDNA synthesis kit
for intact or degraded RNAs
from eukaryotic or bacterial samples
with selectivity for mRNAs and against
rRNAs
suitable for 0.2 to 1 µg total RNA or
for 50 ng of ExpressArt amplified RNA

Catalogue No. 8994-A30
(30 rxns)
ExpressArt® TR cDNA synthesis kit

Reagents
Materials are provided for 30 cDNA synthesis reactions.

**Content Kit box I**
- Tube 1: Primer TR 24 µl
- Tube 2: dNTP-Mix 24 µl
- Tube 3: DEPC-H₂O 1500 µl
- Tube 4: 5x RT Buffer 100 µl
- Tube 5: RNase Inhibitor 10 µl
- Tube 6: RT Enzyme 10 µl

**Content Kit box II**
- cDNA Purification Spin Columns 30 pcs
- Collection Tubes 30 pcs
- Binding Solution, BS 10 ml
- Wash Solution, WS (salt concentrate) 3 ml
- Elution Buffer 10 ml

**Storage**
- **Immediately upon arrival**: store all reagents of **Kit box I at -20°C**. Avoid repeated freeze thawing.
- **Content of Kit box II is stored at room temperature**. Reagents are stable for 9 months.
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Additionally required materials

- For RNA isolation from very small RNA samples, like from laser microdissection or FACS-sorted cells: the ExpressArt RNAready kit which includes ExpressArt NucleoGuard (universal, chemical nuclease inhibitor) and ExpressArt N-Carrier (a small, inert carrier RNA).

- In general, for improved RNA recovery with other commercial spin column kits, we recommend to include the separately available universal, chemical nuclease inhibitor ExpressArt NucleoGuard (#8998-M50).

- For RNA isolation from FFPE (archival, formalin-fixed, paraffin-embedded): the ExpressArt FFPE RNAready kit, which includes a proprietary decrosslinking reagent and ExpressArt NucleoGuard (universal, chemical nuclease inhibitor)

- For improved RNA recovery from FFPE samples with other commercial spin column kits, we recommend to include the separately available ExpressArt FFPE RNA Enhance (#8990-M50), a combination of our universal, chemical nuclease inhibitor ExpressArt NucleoGuard and our chemical decrosslinker DCL.

- Thermocycler. All reactions can be performed in a standard thermocycler (with the lid temperature adjusted to 110°C).

- Pipette-tips (filter-tips recommended)

- Reaction tubes (PCR tubes / 1.5 ml) – do not autoclave!

- 100% Ethanol and 70% 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.
The ExpressArt® FFPE cDNA synthesis kit is suitable for a wide range, from 200 ng to 1 µg of total RNA or for 50 ng of ExpressArt amplified RNA.

1. First strand cDNA synthesis
Prepare First Strand cDNA Synthesis Mix 1, use an appropriate Master mix volume for processing multiple samples.

<table>
<thead>
<tr>
<th>First Strand cDNA Synthesis Mix 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DEPC-H₂O</td>
<td>Tube 3</td>
</tr>
<tr>
<td>dNTP-Mix 10 mM</td>
<td>Tube 2</td>
</tr>
<tr>
<td>Primer TR</td>
<td>Tube 1</td>
</tr>
</tbody>
</table>

Add 4 µl Mix 1 to 4 µl of RNA solution.
Incubate 4 minutes at 65°C in a thermocycler (with heating lid! use standard setting, e.g. 110°C)
Cool samples to 37°C.
In the meantime, prepare the First Strand cDNA Synthesis Mix 2 at room temperature.

<table>
<thead>
<tr>
<th>First Strand cDNA Synthesis Mix 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DEPC-H₂O</td>
<td>Tube 3</td>
</tr>
<tr>
<td>5x RT Buffer</td>
<td>Tube 4</td>
</tr>
<tr>
<td>RNase Inhibitor</td>
<td>Tube 5</td>
</tr>
<tr>
<td>RT Enzyme</td>
<td>Tube 6</td>
</tr>
</tbody>
</table>

Add the First Strand cDNA Synthesis Mix 2 (8 µl) to each sample and mix well by gently flicking the tube.
Incubate the samples in a thermocycler:
37°C / 45 min
45°C / 15 min
50°C / 5 min
70°C / 10 min
4°C / HOLD
Spin briefly to collect liquid.
2. Purification of single-stranded cDNA with Spin Columns

Before starting, add 15 ml of 100% ethanol to the 3 ml Wash Solution concentrate (Kit box II) and mix well.

<table>
<thead>
<tr>
<th>Purification Mix 3</th>
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</thead>
<tbody>
<tr>
<td>Binding Solution (BS) (box II)</td>
</tr>
<tr>
<td>DEPC-H₂O Tube 3</td>
</tr>
</tbody>
</table>

- Add 284 µl of **Mix 3** to each **cDNA Reaction** (16 µl). Mix gently by pipetting.
- 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 Solution BS 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 **Wash Solution WS** (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 **Wash Solution WS**. 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 25 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.
- Repeat the elution step with a second aliquot of 25 µl **Elution Buffer**.
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- The purified template DNA (approximately 48 µl) is now ready for qPCR analysis.
- In general, the cDNA is diluted to 100 µl (with 52 µl water), and 2.5 µl of the diluted cDNA is used per qPCR assay (equivalent amounts of starting material are 5 to 25 ng of total RNA or 1.25 ng of ExpressArt amplified RNA).
- Alternatively, samples can be stored at -20°C for later use.

**Thermocycler profile**
Program a thermocycler with the following temperatures and times. HOLD steps are included to provide time for thermal ramping or for adding reagents.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>65°C</td>
<td>HOLD</td>
<td>Add 4µl Mix1 to 4µl RNA</td>
</tr>
<tr>
<td>65°C</td>
<td>4 min</td>
<td>Renaturing of rRNAs, opening of mRNAs</td>
</tr>
<tr>
<td>37°C</td>
<td>HOLD</td>
<td>add 8µl First Strand cDNA Synthesis Mix 2</td>
</tr>
<tr>
<td>37°C</td>
<td>45 min</td>
<td></td>
</tr>
<tr>
<td>45°C</td>
<td>15 min</td>
<td></td>
</tr>
<tr>
<td>50°C</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>70°C</td>
<td>10 min</td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>HOLD</td>
<td></td>
</tr>
</tbody>
</table>

End of cDNA synthesis
Use cDNA within one day, or **continue** with cDNA purification