

Enhanced RiboAmp™ RNA Amplification Kit (round one)

Box 1: enzymes and mixes (-20°C)

Box 2: columns and buffers (RT)

Co RNA = T-RNA

1st Strand Synthesis

Prepare RNA in 10µl H₂O in a 200µl tube (e.g. 50ng mRNA or not less than 200ng total RNA)

Add 1µl primer **A** (-20°C) (**primer A must be mixed and spun down**)

Heat at 65°C for 5 min

Chill the sample at 4°C for at least 1 min then spin the samples until ready to proceed

(mix and spind down 1st strand reagents)

1 st strand mix	per sample
1 st strand master mix (-20°C)	7µl
1 st strand enzyme mix (-20°C)	2µl
total	9µl

Add 9µl 1st strand mix per sample mix well and spin down

Incubate at 42°C for 45 min (20ul)

Chill the sample to 4°C for at least 1 min

Remove a 2µl aliquot at this point for further quantitative RT-PCR analysis (put in a 0.5ml tube and add 8ul nuclease free water).

Add 2µl of 1st strand nuclease mix (=RNase) (-20°C) (**mix must be mixed and spun down**)

Incubate at 37°C for 20 min (20ul)

Incubate at 95° C for 5 minutes

Put the sample on ice



Sample may be stored at -20°C overnight

2nd Strand Synthesis

Add 1µl of **primer B** (-20°C) (*primer B must be mixed and spun down*)

random hexamers

Incubate at 95°C for 2 min then put the sample on ice for at least 2 minutes

(mix and spin down reagents)

2 nd strand mix	per sample
2 nd strand reaction master mix (-20°C)	29µl
2 nd strand reaction enzyme mix (-20°C)	1µl
total	30µl

Add 30µl 2nd strand mix to the sample mix and spin down

Incubate in the thermo-cycler as follows (program: "sec. strand")

- 25°C 5 min
- 37°C 10 min
- 70°C 5 min
- 4°C hold (up to 30 min)

cDNA purification

Add 250µl of DNA Binding Buffer (**DB**) to a purification column (without sample)

(DNA binding buffer must be mixed)

Hold for 5 min at RT

Spin at 16000 g for 1 min

Discard flow-through

Add 200µl of **DB** to the 2nd strand sample, mix well and pipette the sample into the purification column (gelbe Spitze)

Centrifuge at **100 g** for 2 min, followed by 10.000 g for 30 sec

Discard flow-through

Add 250µl of DNA Wash Buffer (**DW**)

Spin at 16000 g for 2 min

Discard flow-through

Place column into a fresh 1.5ml collection-tube provided in the kit

Add 24 μ l (*neuer Kit 16ul !!*) of DNA Elution Buffer (**DE**)

Incubate for 3 min at RT

Spin at 1000 g for 1 min

Spin at 16000 g for 1 min



You may store the sample at -20°C overnight

Transfer the sample to a new PCR tube (0.2ml)

in vitro Transcription (IVT)

IVT reagents at RT, components should be added in order of the list

(mix and spin down reagents)

IVT reaction mix	per sample
IVT buffer	8 μ l
IVT master mix	12 μ l
IVT enzyme mix	4 μ l
Total	24 μ l

Add 24 μ l of IVT reaction mix to each sample

Incubate at 42°C for 2.5 hours (program: "IVT")

Chill the samples



You may hold the sample(s) at 4°C in the thermal cycler overnight

Add 2 μ l DNase mix ***(mix and spin down)***

Incubate at 37°C for 15 min

Chill the samples

aRNA Purification

Add 250 μ l of RNA Binding Buffer (**RB**) to a new DNA/RNA purification column

(RB must be mixed)

Hold for 5 min at RT

Spin at 16000 g for 1 min

Discard flow-through

Add 200µl of RB to the IVT reaction sample, mix well and pipette the entire sample into the purification column

Centrifuge at 100 g for 2 min, followed by 10.000 g for 30 sec

Discard flow-through

Add 200µl of RNA Wash Buffer (**RW**)

Centrifuge at 10.000 g for 1 min

Discard flow-through

Add 200 µl of **RW**

Spin at 16000 g for 2 min

Discard flow-through

Place the purification column into a new tube (provided in the kit)

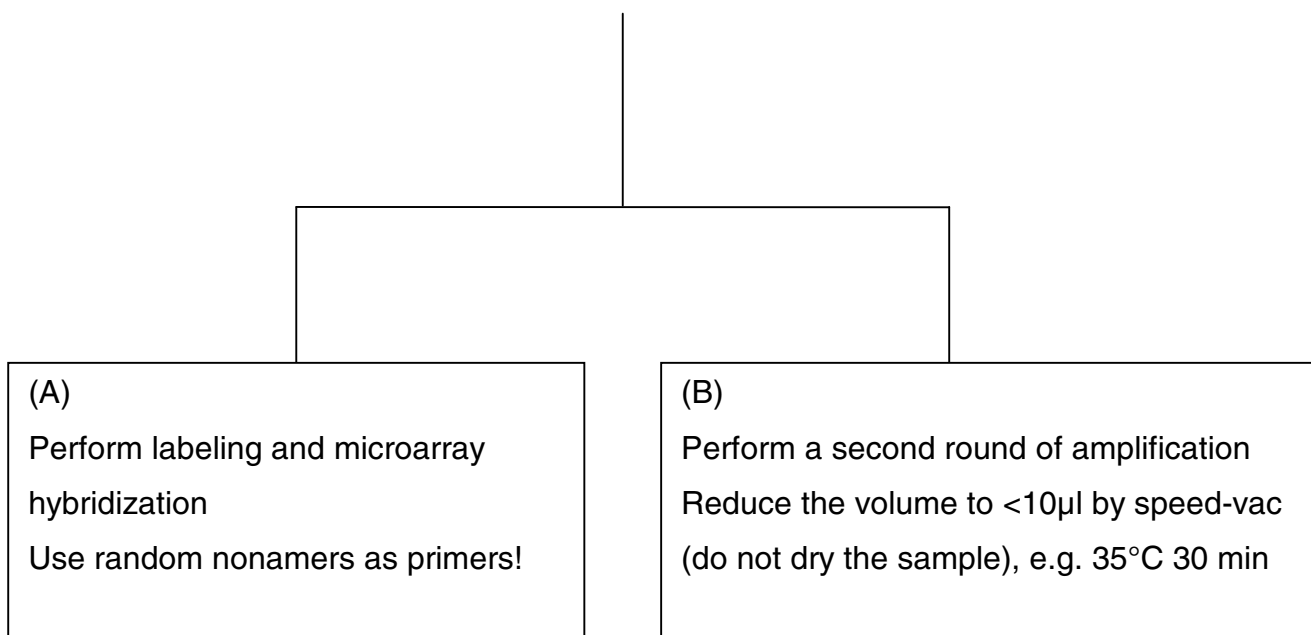
Add 30µl RNA Elution Buffer (**RE**) elution volume=30ul; if a second round is performed use 11ul

Incubate at RT for 3 min

Spin the purification column at 1000g for 1min, followed by 16000 g for 1 min

Measure the $OD_{260/280}$ (dilute 1µl with 49µl TE-Buffer $ph=7.6$)

Store aRNA at -70°C



Enhanced RiboAmp™ RNA Amplification Kit (round two)

Dilute Co aRNA 1:1000

1st Strand Synthesis

Prepare RNA in 10µl H₂O in a new PCR tube

Add **1µl primer B** (-20°C) (*Primer B must be mixed and spun down*)

Heat at 65°C for 5 min

Chill the sample at 4°C for at least 1 min

mix components

1 st strand mix	per sample
1 st strand master mix (-20°C)	7µl
1 st strand enzyme mix (-20°C)	2µl
total	9µl

Add 9µl 1st strand mix per sample mix well and spin down

Incubate at **25°C for 10 min, then at 37°C for 45 min** (20ul, cyclor 1st strand round 2)

Chill the sample to 4°C for at least 1 min (less than 30 min)



Sample may be stored at -20°C overnight

2nd Strand Synthesis

Add **1µl of primer A** (-20°C) (*Primer a must be mixed and spun down*)

Incubate at **95°C for 2 min** then put the sample immediately on ice for 2 minutes
(21ul)

mix components

2 nd strand mix	per sample
2 nd strand reaction master mix (-20°C)	29µl
2 nd strand reaction enzyme mix (-20°C)	1µl
total	30µl

Add **30**µl 2nd strand mix to the sample mix well

Incubate in the thermo-cycler as follows (50µl, 2nd strand round 2)

- **37°C 15 min**
- **70°C 5 min**
- **4°C hold (up to 30 min)**

cDNA purification

Add 250µl of DNA Binding Buffer (**DB**) to a purification column (without sample)

Hold for 5 min at RT

Spin at 16000 g for 1 min

Discard flow-through

Add 200 µl of **DB** to the 2nd strand sample, mix well and pipette the sample into the purification column

Centrifuge at 100 g for 2 min and then at 10000 g for 30 sec

Discard flow-through

Add 250µl of DNA Wash Buffer (**DW**)

Spin at 16000 g for 2 min

Discard flow-through

Place column into a fresh 1.5ml collection-tube provided in the kit

Add **24** ul (*neuer Kit 16 ul!!*) of DNA Elution Buffer (**DE**)

Incubate for 3 min at RT

Spin at 16000 g for 1 min



You may store the sample at -20°C overnight

Transfer the sample to a new PCR tube (0.2 ml)

in vitro Transcription (IVT)

IVT reagents at RT, components should be added in order of the list

mix all reagents

IVT reaction mix	per sample
IVT buffer	8 µl
IVT master mix	12 µl
IVT enzyme mix	4 µl
Total	24 µl

Add **24 µl** of IVT reaction mix to each sample

Incubate at 42°C for **4 hours**

Chill the samples



You may hold the sample(s) at 4°C in the thermal cycler overnight

Add 2 µl DNase mix (must be mixed and spun down)

Incubate at 37°C for 15 min

Chill the samples

aRNA Purification

Add 250µl of RNA Binding Buffer (**RB**) to a new DNA/RNA purification column

Hold for 5 min at RT

Spin at 16000 g for 1 min

Discard flow-through

Add 200 µl of **RB** to the IVT reaction sample, mix well and pipette the entire sample into the purification column

Centrifuge at 100 g for 2 min and then at 10.000 g for 30 sec

Discard flow-through

Add 200µl of RNA Wash Buffer (**RW**)

Centrifuge at 10.000 g for 1min

Discard flow-through

Add 200 μ l of **RW**

Spin at 16000 g for 2 min

Discard flow-through

Place the purification column into a new tube (provided in the kit)

Add **30 μ l** RNA Elution Buffer (**RE**)

Incubate at RT for 3 min

Spin the purification column at 16000 g for 1 min

Measure the $OD_{260/280}$ (dilute 1 μ l with 49 μ l TE-Buffer)



Perform labeling and microarray
hybridization
Use 1 μ l random nonamers as primers!