



Protocol

Reverse Transcription with GeneScript or with GeneScriptPlus

For 20µl RT-Reaction put in the following order

5xGeneScript buffer	4µl
primer	1µl (random-Hexamer 50ng/µl; oligo-dT 0,5µg/µl or 25 pM of a gene specific primer)
DNA polymerization Mix 10 (10mM each dNTP)	2µl (1mM end concentration of each dNTP)
10mM DTT	2µl
RNA template	total RNA – 1-5µg; poly(A) mRNA 1-500ng.
Fill with H₂O to 15µl Incubate in water bath at 80°C for 3min, put on ice and centrifuge	
Rnasin (10U/µl)	2µl
25mM MnCl ₂	2µl

GeneScript or GeneScriptPlus 1µl
 30min at 42°C (GeneScript) or
 incubate 30min at 52°C (GeneScriptPlus)
 10min 80°C
 centrifuge, fill to 100µl with water (“cDNA pool”) – take 1-5µl for PCR using BioTherm™,
 BioThermStar™ or BioThermAB™DNA polymerase.