For life science research only.
Not for use in diagnostic procedures.

Transcriptor High Fidelity cDNA Synthesis Kit

Version: 13
Content Version: February 2021

The Transcriptor High Fidelity cDNA Synthesis Kit is designed to reverse transcribe RNA with an increased fidelity

Cat. No. 05 081 955 001	1 kit 50 reactions, including 10 control reactions
Cat. No. 05 091 284 001	1 kit 100 reactions
Cat. No. 05 081 963 001	1 kit 200 reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function	Catalog Number	Content
1	red	Transcriptor High Fidelity Reverse Transcriptase	Contains Transcriptor Reverse Transcriptase and an enzyme conferring proofreading actifity, 200 mM potassium phosphate, 2 mM dithiothreitol, 0.2% Triton X-100 (v/v), 50% glycerol (v/v), pH approximately 7.2	05 081 955 001	1 vial, 55 µl
				05 091 284 001	1 vial, 110 µl
				05 081 963 001	2 vials, each 110 µl
2	colorless	Transcriptor High Fidelity Reaction Buffer 5x conc.	Contains RT Reaction Buffer, 250 mM Tris/HCl, 150 mM KCl, 40 mM MgCl2, pH approximately 8.5 (+25°C)	05 081 955 001	1 vial, 1 ml
				05 091 284 001	1 vial, 1 ml
				05 081 963 001	2 vials, each 1 ml
3	colorless	Protector RNase Inhibitor	Includes 20 mM Hepes-KOH, 50 mM KCl, 8mM dithiothreitol, 50% glycerol (v/v), pH approximately 7.6 (+4°C)	05 081 955 001	1 vial , 50 µl (40 U/µl
				05 091 284 001	1 vial, 50 µl (40 U/µl)
				05 081 963 001	2 vials, each 50 µl (40 U/µl)
4	purple	Deoxynucleotide Mix 10 mM each dATP, dCTP, dGTP, dTTP		05 081 955 001	1 vial, 200 µl
				05 091 284 001	1 vial, 200 µl
				05 081 963 001	2 vials, 200 µl
5	blue	Anchored-oligo(dT)$_{18}$ Primer		05 081 955 001	1 vial, 100 µl (50 µM)
				05 091 284 001	1 vial, 200 µl (50 µM)
				05 081 963 001	2 vials, each 200 µl (50 µM)
6	colorless	Random Hexamer Primer		05 081 955 001	1 vial, 100 µl (600 µM)
				05 091 284 001	1 vial, 200 µl (600 µM)
				05 081 963 001	2 vials, each 200 µl (600 µM)
7	colorless	DTT			1 vial, 1 ml, 0.1 M
8	colorless	Water, PCR Grade	To adjust the final volume	05 081 955 001	1 vial, 1 ml
				05 091 284 001	2 vials, each 1 ml
				05 081 963 001	3 vials, each 1 ml
9	green	Control RNA	Contains a stabilized solution of a total RNA fraction purified from an immortalized cell line (K562)	05 081 955 001	1 vial, 20 µl (50 ng/µl)
10	green	Control Primer Mix PBGD	Human porphobilinogen deaminase (PBGD) forward and reverse primer to amplify a 151 bp fragment	05 081 955 001	1 vial, 40 µl, (5 µM)

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Cap	Label	Storage
1	red	Transcriptor High Fidelity Reverse Transcriptase	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	colorless	Transcriptor High Fidelity Reaction Buffer, 5x conc.	
3	colorless	Protector RNase Inhibitor	
4	purple	Deoxynucleotide Mix, 10 mM each dATP, dCTP, dGTP, dTTP	
5	blue	Anchored-oligo(dT)$_{18}$ Primer	
6	colorless	Random Hexamer Primer	
7	colorless	DTT	
8	colorless	Water, PCR Grade	
9	green	Control RNA	Store at −60°C or below. ⚠ ***Avoid repeated freezing and thawing.***
10	green	Control Primer Mix PBGD	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions
- Standard benchtop microcentrifuge

For the RT-PCR reaction

- Conventional block cycler with heated lid
- PCR reaction vessels (thin-walled PCR tubes or plates are recommended)
- Template RNA
- Sequence-specific PCR primers (optional)

For control reactions in combination with a LightCycler® Instrument:

- LightCycler® Carousel-Based System or LightCycler®480 Instrument

1.4. Application

The Transcriptor High Fidelity cDNA Synthesis Kit is designed to reverse transcribe RNA with an increased fidelity compared to other reverse transcriptases. The kit features Transcriptor High Fidelity Reverse Transcriptase, a blend of a recombinant reverse transcriptase and a proofreading mediating enzyme optimized for two-step RT-PCR, making this the product of choice for the following applications:

- Cloning genes of interest
- Sequencing transcriptomes
- Generating cDNA libraries with large and full-length inserts
- Study gene expression levels requiering high fidelity, such as RNA splicing analysis via two-step RT-PCR, using qualitative RT-PCR on conventional thermal cyclers or quantitative RT-PCR on the LightCycler® Carousel-Based System, the LightCycler® 480 System, or other real-time PCR instruments.

The kit contains all components required for synthesizing cDNA suitable for direct use in qualitative RT-PCR with conventional thermal cyclers or quantitative RT-PCR on real-time PCR instruments. The 50-reaction pack size also includes 10 control reactions (control RNA and control primer mix).

Product Description

The core component of the Transcriptor High Fidelity cDNA Synthesis Kit is the Transcriptor High Fidelity Reverse Transcriptase, an enzyme blend of a recombinant reverse transcriptase and a proofreading mediating enzyme. The synergy between both enzymes is the key to the ability of the enzyme blend to reverse transcribe RNA templates with a sevenfold higher fidelity compared to other commonly used reverse transcriptases. Due to the high thermostability of both Transcriptor High Fidelity enzyme components and the specially optimized buffer system, reverse transcription is possible at temperatures up to +55°C. This allows the reverse transcription of GC-rich templates with high secondary structure, without including additives that can interfere with the reverse transcription reaction.

1.5. Preparation Time

Assay Time

around 1 hour

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Tempate RNA: Isolated total RNA, mRNA, viral RNA or *in vitro* transcribed RNA.
Use 1 ng up to 4 µg RNA. If the RNA concentration in the template preparation is low, stabilize RNA by adding MS2RNA (10 µg/ml) to the preparation.

⚠ ***High quality intact RNA, free of residual genomic DNA, RNase, and inhibitors is essential for good results. In particular, take the following precautions to avoid contaminating RNA with RNase at any step in the isolation process (starting with cell lysis):***

- Use either RNase inhibitors such as Protector RNase Inhibitor or isolation conditions that inactivate RNases.
- If necessary, analyze different steps in the process (*e.g.*, lysis, isolation) by gel electrophoresis (ethidium bromide staining) to ensure that the sample is RNase-free.
- Remember that RNases can also be present on contaminated glassware.

ⓘ *To prepare total RNA or mRNA, we recommend using Roche Diagnostics reagents. For information on automated nucleic acid isolation using the MagNA Pure Systems, visit www.magnapure.com.*

Control Reactions

The control reaction which is provided in Cat.No. 05081955001 includes reverse transcription of the Control RNA followed by detection of a 151 bp fragment of PBGD in a PCR on a conventional thermal block cycler or a LightCycler® Instrument.

Primers

Depending on the type of analysis, to which the cDNA is to be subjected, use one of two different priming methods described below:

Anchored-oligo(dT)18 primer

- As anchored-oligo$(dT)_{18}$ primers are specific to the small pool of poly(A)+ RNA in the whole total RNA pool (1 to 2%), the amount of cDNA resulting from reverse transcription reactions with anchored-oligo$(dT)_{18}$ primers is considerably lower than with random hexamers. Anchored-oligo$(dT)_{18}$ priming is recommended when performing RT-PCR for new mRNA targets. Anchored-oligo$(dT)_{18}$ produces an RT-PCR product more consistently than random hexamers or gene-specific primers.
- Hybridizes to the very beginning of the poly(A) tail.
- Prevents priming from internal sites of the poly(A) tail.
- Generates full-length cDNA.
- Preferred priming method for most two-step RT-PCR.
- Available as part of the Transcriptor First Strand cDNA Synthesis Kit only.

Random hexamer primer

- For most applications, reverse transcribe 5 µg total RNA using random hexamers at a final primer concentration of 60 µM. Increasing the concentration of hexamers to higher concentrations for the transcription of 5 mg RNA may increase yield of small PCR products (< 500 bp), but may decrease the yield of longer PCR products and full-length transcripts.
- Hybridizes to many sites throughout the length of an RNA.
- Provides uniform representation of all RNA sequences in mRNA.
- Can prime cDNA transcription from RNAs that do not carry a poly(A)+ tail.
- The ratio of random primers to RNA in the RT reaction determines the average length of cDNAs generated. Example: A high ratio will generate relatively short cDNAs, increasing the chance of copying the entire target sequence.
- Short cDNA transcripts may help to overcome difficulties caused by RNA secondary structures.

General Considerations

Precautions

Take special precautions when working with RNA:

- Always wear gloves when working with RNA. After putting on gloves, do not touch surfaces and equipment to avoid reintroduction of RNases to decontaminated material.
- Designate a special area for RNA work only and if possible use reaction vessels and pipettors dedicated only for work with template RNA.
- Treat surfaces of benches and glassware with commercially available RNase inactivating agents. Clean benches with 100% ethanol.
- Use commercially available sterile and RNase-free disposable plasticware only.
- Purchase reagents that are free of RNases. Reserve separate reagents for RNA work only. Use DEPC-treated water for all solutions.
- Keep all reagents on ice.
- Extract RNA as quickly as possible after obtaining samples. For best results, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at −60°C or below.

Incubation Temperature / TIME

Depending on the RNA target chosen, the optimal reaction temperature and incubation time will vary. Transcriptor High Fidelity Reverse Transcriptase can be used at temperatures between +45°C and +55°C, also for GC-rich targets. The recommended incubation time is 30 minutes. For many reactions however 10 minutes are sufficient.
The cDNA can be used for amplification without further purification or manipulation such as RNase H-treatment. For PCR use 1 to 5 µl of the reaction product in 20 µl or 50 µl final reaction volume.

Prevention of Carryover Contamination

DNA Contamination

Include appropriate positive and negative control reactions to exclude artifacts from DNA targets, such as residual genomic DNA contaminations from RNA preparations or contaminating DNA from previous amplifications.

2.2. Protocols

Standard Procedure for Qualitative RT-PCR

The following conditions describe a first-strand cDNA synthesis for a two-step RT-PCR.
Fig. 1a shows a flow-diagram of the standard procedure for cDNA synthesis with anchored-oligo $(dT)_{18}$ primer or random hexamer primer for qualitative RT-PCR.

anchored-oligo $(dT)_{18}$ priming O

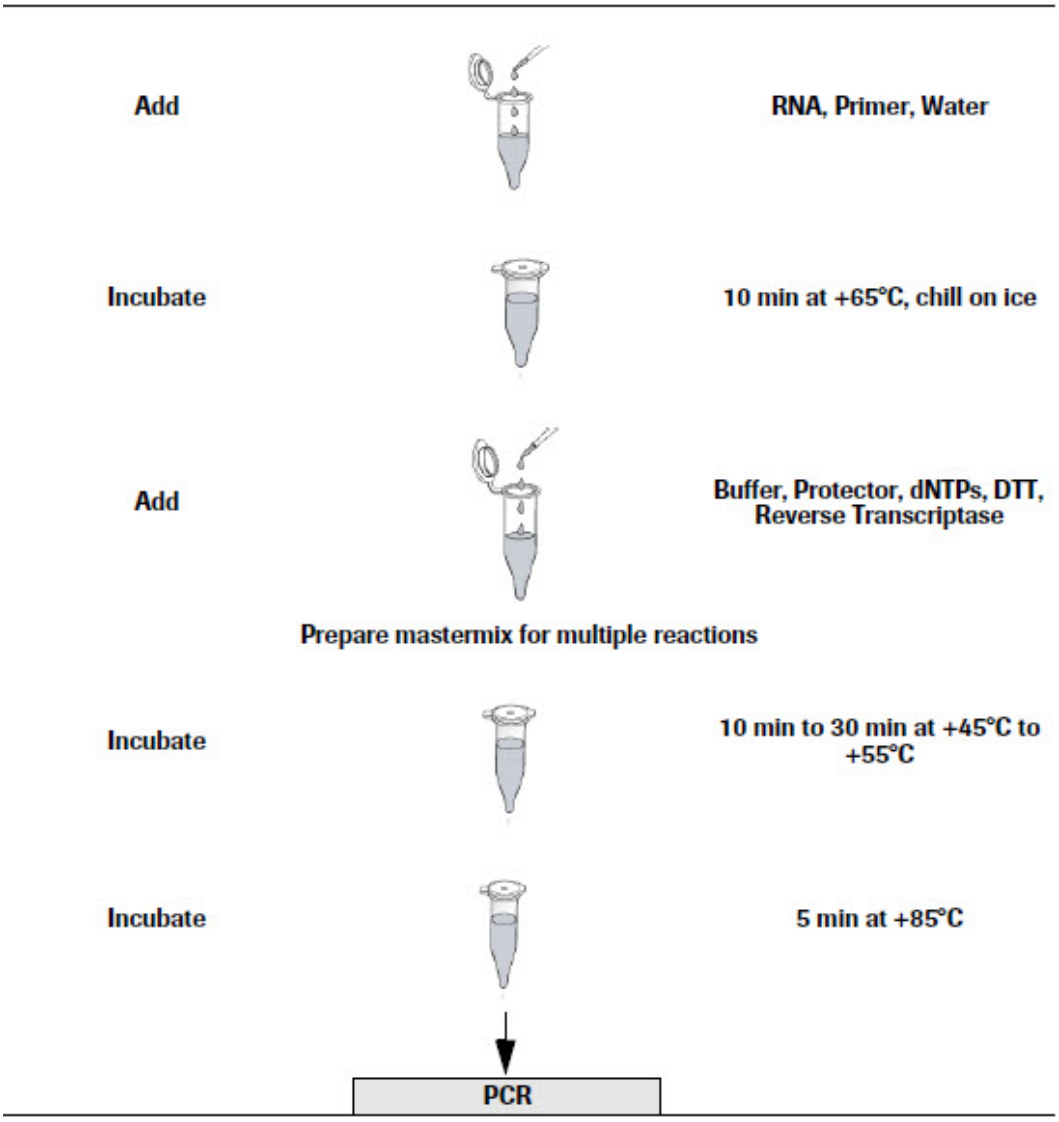

Fig. 1a: Overview of cDNA synthesis procedures in single and multiple reactions

Reverse transcription using either anchored-oligo $(dT)_{18}$ priming OR random hexamer priming.

In the major cases, cDNA is generated with only one sort of primers.

1. Thaw the components listed below and place them on ice.

2. Briefly centrifuge all reagents before setting up the reactions.

3. Set up the reaction components in a nuclease free microcentrifuge tube placed on ice

Reagent	Volume	Final conc.
total RNA	variable	1 ng up to 4 µg[1)]
Primer -choose either		
Anchored-oligo(dT)18 Primer, 50 pmol/ml (vial 5)	1 µl	2,5 µM
OR Random Hexamer Primer, 600 pmol/ml (vial 6)	2 µl	60 µM
Water, PCR Grade (vial 8)	variable	
Total	**11,4 µl**	

1) When working with low concentrated RNA samples (< 10 µg/ml), add 10 µg/ml MS2 RNA* to stabilize the template RNA.

4. Mix the reagents and centrifuge briefly to collect the sample at the bottom of the tube.

5. Denature the template-primer mixture by heating the tube for 10 minutes at +65°C in a block cycler with a heated lid (to minimize evaporation). This step ensures denaturation of RNA secondary structures.

6. Immediately cool the tube on ice.

7. To the tube containing the template-primer mix, add the remaining components of the RT mix in the order listed below.
   There is also the possibility to prepare master mix for multiple reactions.

Reagent	Volume	Final conc.
Transcriptor High Fidelity Reverse Transcriptase Reaction Buffer, 5x conc. (vial 2)	4 µl	1x 8 mM $MgCl_2$
Protector RNase Inhibitor, 40 U/ml (vial 3)	0,5 µl	20 U
Deoxynucleotide Mix, 10 mM each (vial 4)	2 µl	1 mM each
DTT (vial 7)	1 µl	5 mM
Transcriptor High Fidelity Reverse Transcriptase (vial 1)	1,1 µl	22 U
Total	**20 µl**	

9. Mix the reagents in the tube carefully and centrifuge briefly to collect the sample at the bottom of the tube.

10. Place the tube in a block cycler with heated lid (to minimize evaporation)

11 Incubate the reaction for 10 to 30 minutes at +45°C to +55°C. Depending on the RNA target chosen, optimal reaction temperature and time may vary. Transcriptor High Fidelity Reverse Transcriptase can be used for temperatures between +45°C and +55°C, also for GC-rich targets. The recommended incubation time is 30 minutes. For many reactions however 10 minutes are sufficient.

⚠ ***Preheat the block cycler to the temperature of the RT reaction***

12 Inactivate Transcriptor High Fidelity Reverse Transcriptase by heating to +85°C for 5 minutes. Stop the reaction by placing the tube on ice.

At this point the reaction tube may be stored at +2 to +8°C for 1 to 2 hours or at -15 to -25°C for longer periods. The cDNA can be used for amplification without further purification or manipulation such as RNase H-treatment. For PCR use 1 to 5 µl of the reaction product in 20 µl or 50 µl final reaction volume.

Standard Procedure for Qualitative RT-PCR

The following conditions describe a first-strand cDNA synthesis for a two-step RT-PCR.
Fig. 1b shows a flow-diagram of the standard procedure for cDNA synthesis with random hexamer primers for quantitative RT-PCR.

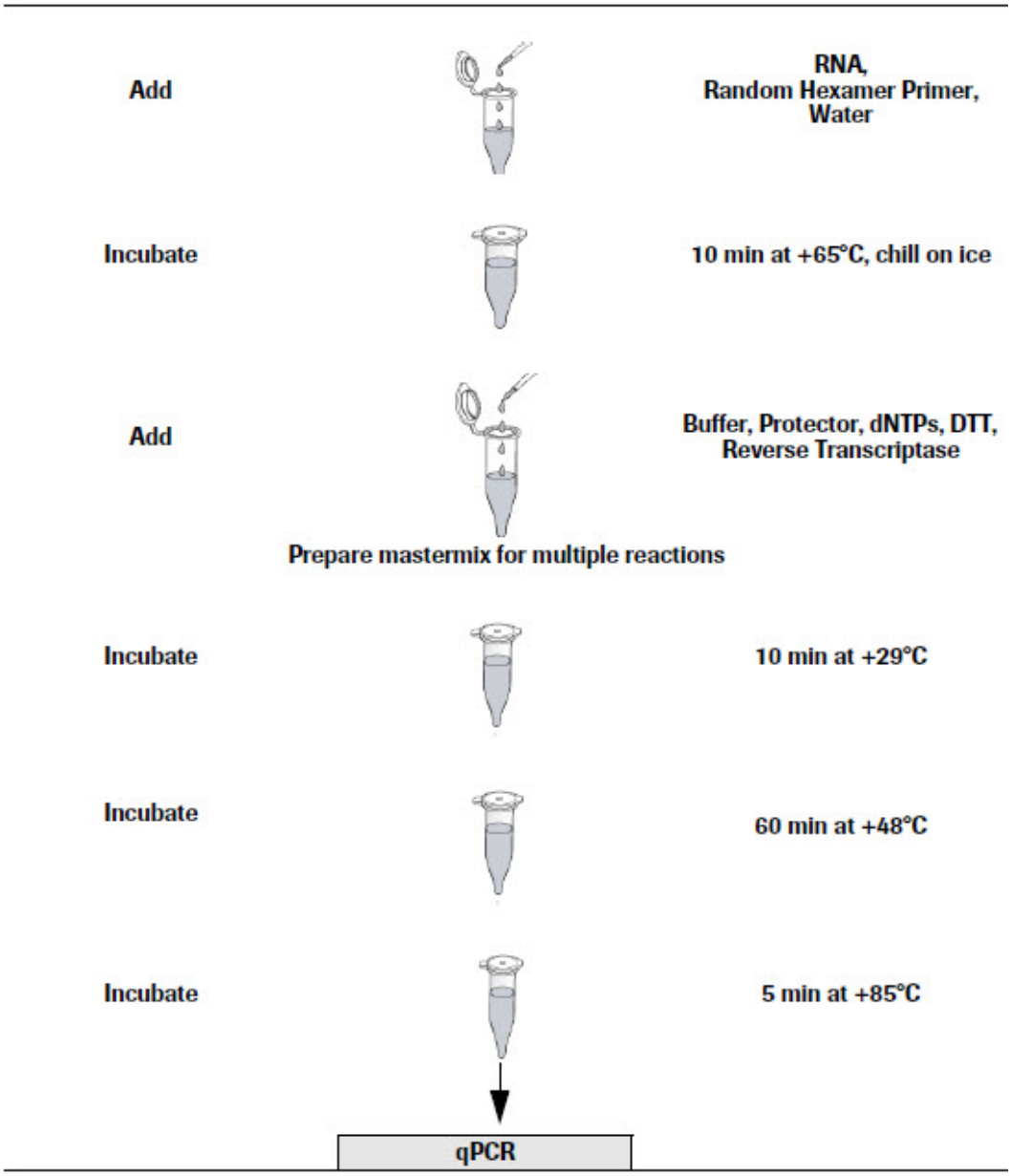

Fig. 1b: Overview of cDNA synthesis procedures in single and multiple reactions

Reverse Transcription using random hexamer priming

1. Thaw the components listed below and place them on ice.

2. Briefly centrifuge all reagents before setting up the reactions.

3. Set up the reaction components in a nuclease free microcentrifuge tube placed on ice:

Template-Primer Mix (for 1 reaction)		
Reagent	**Volume**	**Final conc.**
total RNA	variable	1 pg up to 1 µg[1)]
Random Hexamer Primer, 600 pmol/ml (vial 6)	2 µl	60 µM
Water, PCR Grade (vial 8)	variable	
Total	**11,4 µl**	

1) When working with low concentrated RNA samples (< 10 µg/ml), add 10 µg/ml MS2 RNA* to stabilize the template RNA.

4. Mix the reagents in the tube carefully and centrifuge briefly to collect the sample at the bottom of the tube.

5. Incubate for 10 minutes at +65°C, in a block cycler with a heated lid (to minimize evaporation). This step ensures denaturation of RNA secondary structures.

6. Immediately cool the tube on ice.

7. To the tube containing the template-primer mix, add the remaining components of the RT mix in the order listed below. *There is also the possibility to prepare master mix for multiple reactions.*

Reagent	**Volume**	**Final conc.**
Transcriptor High Fidelity Reverse Transcriptase Reaction Buffer, 5× conc. (vial 2)	4 µl	1x 8 mM MgCl2
Protector RNase Inhibitor, 40 U/ml (vial 3)	0,5 µl	20 U
Deoxynucleotide Mix, 10 mM each (vial 4)	2 µl	1 mM each
DTT (vial 7)	1 µl	5 mM
Transcriptor High Fidelity Reverse Transcriptase (vial 1)	1,1 µl	22 U
Total	**20 µl**	

8. Mix the reagents in the tube carefully and centrifuge briefly to collect the sample at the bottom of the tube.

9. Place the tube in a block cycler with heated lid (to minimize evaporation)

10. Incubate the reaction for 10 minutes at +29°C. Then incubate for 60 minutes at + 48°C.
⚠ ***Preheat the block cycler to the temperature of the RT reaction***

11. Inactivate Transcriptor High Fidelity Reverse Transcriptase by heating to +85°C for 5 minutes. Stop the reaction by placing the tube on ice.

At this point the reaction tube may be stored at +2 to +8°C for 1 to 2 hours or at -15 to -25°C for longer periods. The cDNA can be used for amplification without further purification or manipulation such as RNase H-treatment. For PCR use 1 to 5 µl of the reaction product in 20 µl or 50 µl final reaction volume.

Setup of the Control Reaction

cDNA Synthesis

The control reaction which is provided includes reverse transcription of the Control RNA followed by detection of a 151 bp fragment of PBGD in a PCR on a conventional block cycler or a LightCycler® Instrument.

1. Thaw the components listed below and place them on ice.

2. Vortex briefly and centrifuge all reagents before setting up the reactions.

3. Set up the control reaction in a nuclease free microcentrifuge tube placed on ice:

Template -Primer Mix (for 1 reaction)		
Reagent	**Volume**	**Final conc.**
Control RNA	2 µl	100 pg
Anchored-oligo $(dT)_{18}$primer, 50 pmol/µl	1 µl	
Water, PCR Grade	8,4 µl	
Total volume	11,4 µl	

4. Incubate for 10 minutes at +65°C, chill on ice

5. Add the following components

Reagent	**Volume**	**Final conc.**
Transcriptor High Fidelity Reverse Transcriptase Reaction Buffer, 5× conc. (vial 2)	4 µl	1x 8 mM $MgCl_2$
Protector RNase Inhibitor, 40 U/ml (vial 3)	0,5 µl	20 U
Deoxynucleotide Mix, 10 mM each (vial 4)	2 µl	1 mM each
DTT (vial 7)	1 µl	5 mM
Transcriptor High Fidelity Reverse Transcriptase (vial 1)	1,1 µl	22 U
Total	**20 µl**	

6. Mix well by pipetting and centrifuge briefly to collect the sample at the bottom of the tube.

7. Incubate 30 minutes at +55°C

8. Inactivate Transcriptor High Fidelity Reverse Transcriptase by heating to +85°C for 5 minutes.

9. Place the tube on ice.

10. At this point the reaction tube may be stored at +2°C to +8°C or at -15 to -25°C

PCR for PBGD

The resulting single-stranded cDNA can be amplified in a polymerase chain reaction utilizing the supplied PBGD-specific primers. This PCR may be done on a conventional block cycler or on a LightCycler® Instrument:

- Use 5 µl of the cDNA reaction for PCR on a conventional block cycler with a reaction volume of 50 µl using FastStart Taq DNA Polymerase*.
- Use 2 µl of the cDNA reaction for real-time PCR on the LightCycler® 1.5 Instrument or LightCycler® 2.0 Instrument with a reaction volume of 20 µl using the LightCycler® FastStart DNA Master SYBR Green I*.

⚠ ***When performing PCR experiments using the LightCycler® instruments, program an initial 10 minutes denaturation step for FastStart Polymerase activation.***

For further details of the PCR or real-time PCR read the Instructions for Use of FastStart Taq DNA Polymerase, LightCycler® FastStart DNA Master SYBR Green I, or LightCycler® 480 SYBR Green I Master.

The following conditions describe the first-strand cDNA synthesis for a two-step control RT-PCR.N Preheat the block cycler to the temperature of the RT reaction (see step 4 below) before starting the procedure.
Setup of the PCR in a conventional block cycler

1. Thaw the components listed below and place them on ice.

2. Vortex briefly and centrifuge all reagents before setting up the reactions.

3. Set up the control reaction in a nuclease free, thin-walled PCR tube placed on ice in the order listed below:

Reagent	Volume	Final conc.
FastStart buffer with 20 mM $MgCl_2$ (10x)	5 µl	1x
PCR Nucleotide Mix *(10 mM)	1 µl	0.2 mM
Control Primer Mix PBGD (5 µM)	2 µl	0.2 µM
cDNA from Control RT reaction	5 µl	
FastStart Taq DNA Polymerase* (5U/µl)	0.4 µl	2 U
Water, PCR Grade	36.6 µl	
Total	**50 µl**	

4. RUN PCR in a block cycler

Step	Action			
Sample loading	Overlay the reaction with 30 µl mineral oil, if required by the type of block cycler used.			
Initial denaturation	94°C for 5 min			
Standard PCR Profile	**Setup**	**Temp.**	**Time [s]**	**Cycles**
	Denaturation	94°C	10	35
	Annealing	50°C	20	
	Elongation	72°C	30	
	Final elongation	72°C	7 min	1
	Cooling	4°C		
Analyze samples	Load 15 µl on a 3% agarose gel containing Ethidium bromide. The PCR product has a size of 151 bp			

PCR on the LightCycler® Carousel-Based System The Control Primer Mix PBGD enclosed can be used for amplification of a 151 bp fragment detected in the SYBR Green format.
Perform a negative control reaction without template (using water instead of cDNA) in parallel to the RT control reaction.
Perform PCR with 5 µl cDNA and LC FastStart DNA MasterPLUS SYBR Green I* (Cat. No. 03 515 869 001) according to the procedures within the Instructions for Use.

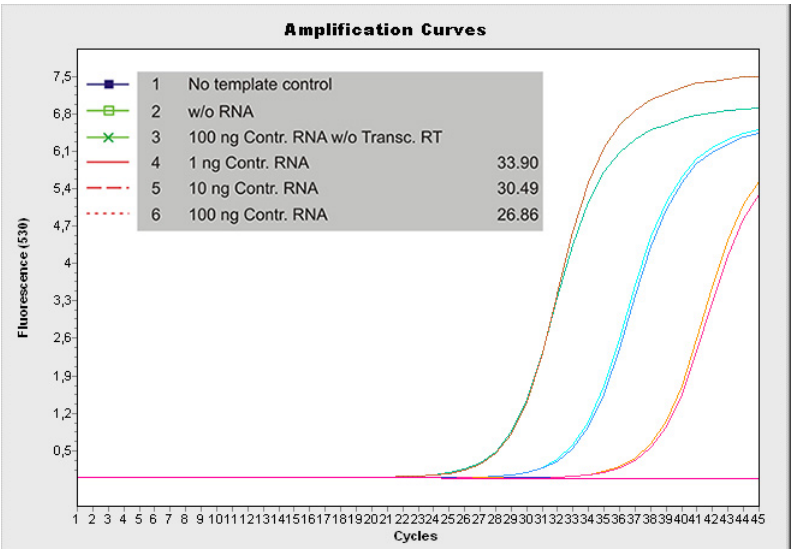

Fig. 2: Linear control reaction:
Control reaction with PBGD primers using LightCycler® FastStart DNA MasterPLUS SYBR Green I for the LightCycler® Carousel-Based Instrument reaction. The supplied Control RNA was used in different dilutions.

2.3. Parameters

Accuracy

7-fold higher compared to conventional RT-PCR
Error rate was determined by sequencing using the Genome Sequencer 20 System. RNA was reverse transcribed with the Transcriptor High Fidelity Reverse Transcriptase and a MMLV reserve transcriptase. After cDNA purification and amplification with a proofreading polymerase, the error rate was calculated by subtracting the error rate of the PCR control performed with plasmid DNA carrying the same sequence. The error rate of the Transcriptor High Fidelity Reverse Transcriptase is a mean value of 4 independent experiments in which at least 3.1×10^6 bases were sequenced. For the MMLV reverse transcriptase, 4.5×10^6 bases were sequenced.
Pwo SuperYield and Taq Polymerase fidelity was also determined by GS20 sequencing. From the Errors in PCR the fidelity was calculated using the formula:
(number of errors/total bases sequenced minus background) /d, where d is the number of DNA duplications calculated by the formula: 2^d = yield/input DNA.

Detection range

10 pg total RNA

Inactivation

+85°C for 5 minutes.

Maximum Fragment Size

Up to 14 kb

Speed

10 to 30 minutes
reverse transcription

3. Results

Product length and yield

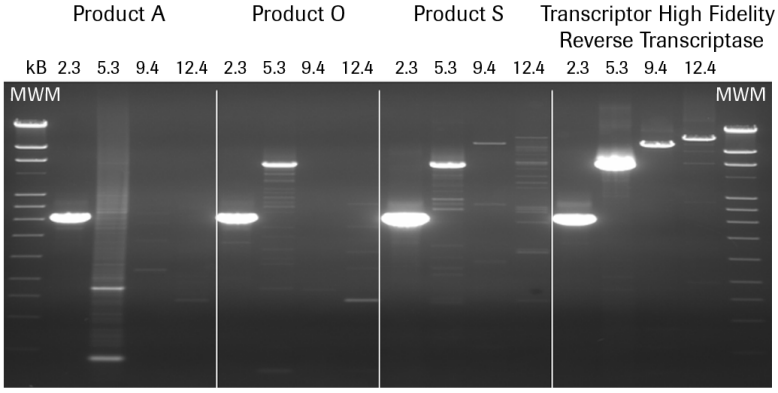

Fig. 3: Comparison of different reverse transcriptases for the reverse transcription of total RNA for different fragment sizes.
1 g (2.3 kb, 5.3 kb and 9.4 kb) or 2 g (12.4 kb) of total RNA were transcribed according to manufacturers recommendations. 5 l from the cDNA reaction were subsequently amplified with Expand Long Template PCR System*. RNA sources used: human muscle for the 2.3 kb fragment, HeLa cells for the 5.3 kb and 9.4 kb fragment, and rat brain for the 12.4 kb fragment.

Temperature for cDNA Synthesis

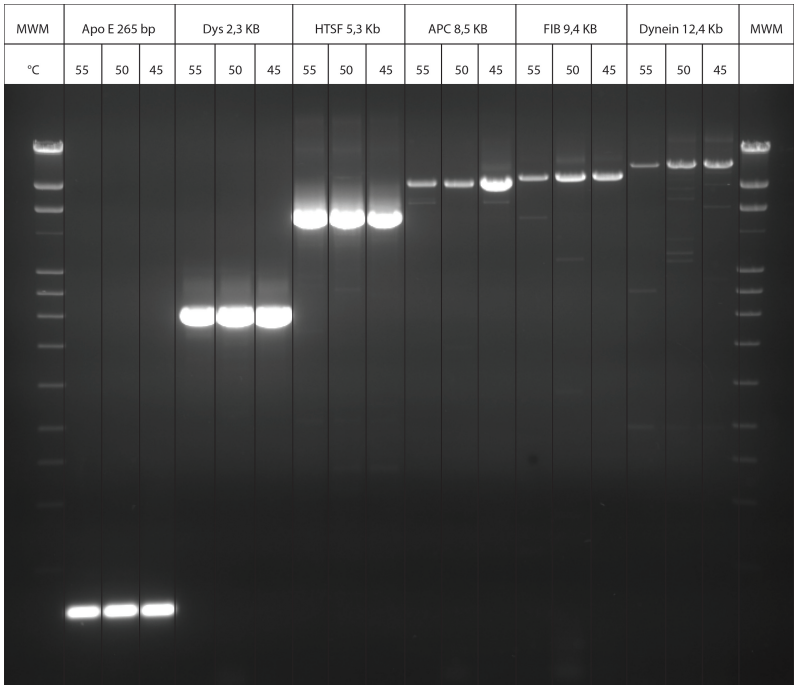

Fig. 4: Comparison of different temperatures for cDNA synthesis
cDNA synthesis with Transcriptor High Fidelity cDNA Synthesis Kit was performed at +55°C, +50°C and +45°C on 1 g of total RNA for 30 min. 5 l from the cDNA reaction were subsequently amplified with Expand Long Template PCR System*. RNA sources used: human liver for Apo E, human muscle for Dystrophin, HeLa cells for HTSF, APC and FIB and rat brain for Dynein.

Incubation time for cDNA reaction

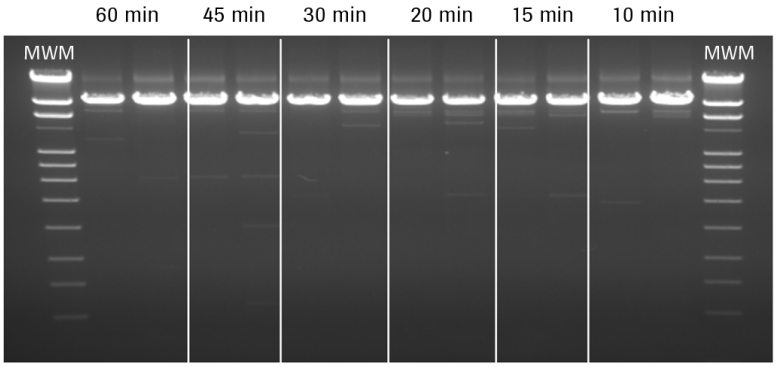

Fig. 5: Comparison of different incubation times for cDNA reactions, using the Transcriptor High Fidelity cDNA Synthesis Kit.
One microgram of HeLa total RNA was reverse transcribed at 50°C for the times indicated. Five-microliter aliquots of the cDNA reactions were amplified with primers for an 8.5 kb fragment, using Roche™s Expand Long Range dNTPack. All reactions were performed in duplicate. Results show that the Transcriptor High Fidelity cDNA Synthesis Kit efficiently transcribes RNA with high speed and accuracy.

Dynamic Range in 2-Step qRT-PCR

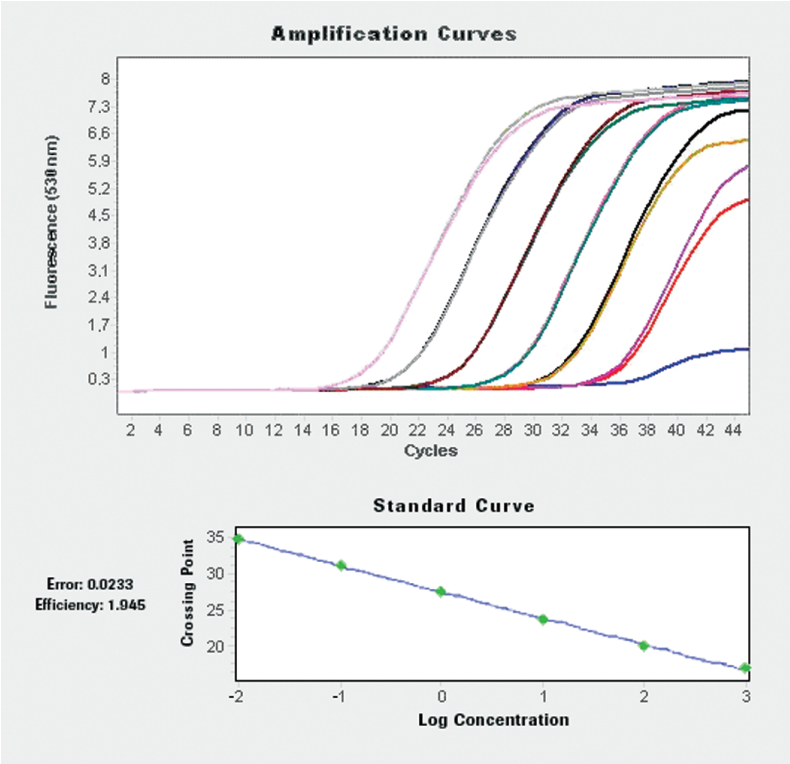

Fig. 6: Example of anchored-oligo(dT) - primer
1 µg to 10 pg of K562 total RNA were reverse transcribed with anchored-oligo(dT) – primer in 20 µl volume for 30 min at 55°C. 5 µl from each reaction were amplified with LightCycler® TaqMan Master*, primers for ß-actin and UPL probe No.11*.

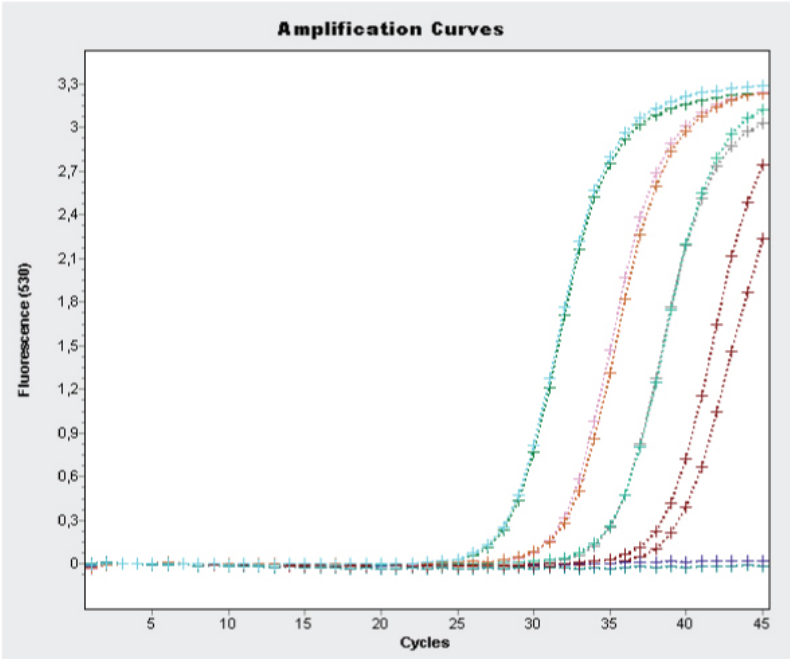

Fig. 7: Example of random hexamer primer
10 ng to 10 pg of total RNA from HeLa cells were reverse transcribed with random hexamer primer for 30 min at +50°C. 5 l of cDNA were amplified with LightCycler® FastStart DNA MasterPLUS HybProbe*, primer and hydrolysis.

4. Troubleshooting

Observation	Possible cause	Recommendation
No PCR product or very little amount of PCR product	Insufficient amount of template RNA.	Check quality and concentration of template.
		Use poly(A)+ mRNA rather than total RNA as template.
		Increase amount of RNA template in cDNA reaction. Use 10 ng to 5 µg of total RNA or 1 to 100 ng mRNA.
	Template RNA degraded.	Prepare fresh RNA template, being careful to prevent RNase activity.
		Check RNA preparation by gel electrophoresis.
	Too much template RNA.	A too high amount of template RNA may affect/inhibit performance of RT-PCR; decrease amount of RNA template.
	RT-PCR Inhibitors are present in the RNA.	Make sure that the RNA is free of RT-PCR inhibitors, such as by using Roche High Pure or MagNA Pure Kits for RNA purification and isolation.
	Reaction not optimized.	Increase primer concentration (up to 1°C M maximum).
		Synthesize the cDNA for 30 minutes at a temperature between +42 and +60°C. *Prolonged incubation at lower temperatures will increase the yield of full-length product. Use 60 minutes incubation time.*
	Enzyme concentration too high or too low	Do not use more than 1.1 µl Transcriptor High Fidelity Reverse Transcrptase to transcribe 1 µg total RNA template in a 20 µl cDNA synthesis reaction
		For > 5 µg total RNA, increase reaction volume and enzyme amount proportionally.
	Template secondary structure prevented effective first strand cDNA synthesis.	Raise temperature for reverse transcription reaction up to +60°C or use Transcriptor Reverse Transcriptase to reverse transcribe at temperature as high as +65°C.
	Template secondary structure inhibits effective formation of full-length products.	If GC content of RNA is high (>60%), increase denaturation temperature or denaturation time in PCR cycles.
No PCR product or very little amount of PCR product	Incubation temperature too high.	For higher reverse transcription reaction temperatures, primers with appropriate melting temperatures must be used. The annealing temperature in PCR depends on the melting temperature of the respective primer pair. Use an appropriate computer program to calculate the optimal temperature for the primers used. The recommended annealing temperature is the melting temperature of the primers or 2°C below.
	Use of Random Hexamer primer	The ratio of random primers to RNA can be adjusted to control the average length of cDNA products; high ratios as recommended in these Instructions for Use will produce shorter cDNAs, but should increase the likelihood of copying the target sequence (fragments up to 6 kb were amplified by PCR using the recommended conditions). If longer cDNAs are needed, the concentration of random primers may be decreased down to 1.5 mM in the cDNA synthesis reaction.
	Too much cDNA inhibits PCR	The volume of cDNA template (from the RT reaction) should not exceed 10% of the total volume of the PCR reaction.

Background smear	Secondary amplification product(s).	Check reagent concentrations and cycling conditions:
		Optimize temperature of cDNA synthesis step.
		Optimize primer concentration.
		Decrease number of cycles.
		Check and perhaps decrease concentration of template.
		Optimize $MgCl_2$ concentration of the PCR reaction and for each template and primer combination *The final $MgCl_2$ concetnration in the reverse transcription is 8 mM. Therefore, each µl of the cDNA contributes 0.16 mM $MgCl_2$ to the reaction.*
Nonspecific product bands	Annealing temperature too low.	Increase annealing temperature during PCR to increase specificity of amplification.
	Primer-dimers formed.	Design primers without complementary sequences at the 3' ends.
		Make sure a denaturation step is included at the end of the cDNA synthesis reaction (5 minutes at +85°C)
	Contaminating DNA in sample.	Perform a control without reverse transcription step.
		Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating DNA.

5. Additional Information on this Product

5.1. Quality Control

Each lot of the Transcriptor High Fidelity cDNA Synthesis Kit is function tested in an assay to quantify proofreading activity and in a two-step qRT-PCR assay to quantify the presence of PBGD mRNA in total RNA from K562 cells.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Information related to the REACH Annex XIV removed.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit, 96 reactions of 20 µl final volume each	03 515 869 001
	1 kit, 480 reactions of 20 µl final volume each	03 515 885 001
	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 186 001
PCR Nucleotide Mix^{PLUS}	2 x 100 µl, 200 PCR reactions in 50 µl	11 888 412 001
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001
High Pure RNA Isolation Kit	1 kit, 50 isolations	11 828 665 001
High Pure Viral RNA Kit	1 kit, up to 100 purifications	11 858 882 001
Titan One Tube RT-PCR Kit	1 kit, 50 reactions including 10 control reactions	11 939 823 001
RNA, MS2	500 µl, 10 A_{260} units	10 165 948 001
LightCycler® FastStart DNA Master SYBR Green I	1 kit, 96 reactions of 20 µl final volume each	03 003 230 001
	1 kit, 480 reactions of 20 µl final volume each	12 239 264 001
High Pure RNA Tissue Kit	1 kit, 50 isolations	12 033 674 001
LightCycler® FastStart DNA Master HybProbe	1 kit, 96 reactions of 20 µl final volume each	03 003 248 001
	1 kit, 480 reactions of 20 µl final volume each	12 239 272 001
Titan One Tube RT-PCR System	100 µl, 100 reactions	11 855 476 001
LightCycler® TaqMan® Master	1 kit, 96 reactions of 20 µl final volume each	04 535 286 001
	1 kit, 480 reactions of 20 µl final volume each	04 735 536 001
mRNA Isolation Kit	1 kit	11 741 985 001
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
5′/3′ RACE Kit, 2nd Generation	1 kit, 10 reactions	03 353 621 001
High Pure RNA Paraffin Kit	1 kit, up to 100 isolations	03 270 289 001
High Pure FFPET RNA Isolation Kit	1 kit, 50 isolations	06 650 775 001
LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit, 96 reactions of 20 µl final volume each	03 515 575 001
	1 kit, 480 reactions of 20 µl final volume each	03 515 567 001
	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 178 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U, 200 reactions of 20 µl final volume	03 531 287 001
Expand Long Template PCR System	150 U, 1 x 150 U, 38 reactions in a final volume of 50 µl	11 681 834 001
	720 U, 2 x 360 U, 190 reactions in a final volume of 50 µl	11 681 842 001
	3,600 U, 10 x 360 U, 950 reactions in a final volume of 50 µl	11 759 060 001
FastStart High Fidelity PCR System	500 U, 2 x 250 U, 5 U/µl, 200 reactions in a final volume of 50 µl	03 553 400 001
	2,500 U, 10 x 250 U, 5 U/µl, 1,000 reactions in a final volume of 50 µl	03 553 361 001

FastStart Taq DNA Polymerase, 5 U/µl	100 U, 1 x 100 U, 50 reactions in a final volume of 50 µl	12 032 902 001
	500 U, 2 x 250 U, 250 reactions in a final volume of 50 µl	12 032 929 001
	1,000 U, 4 x 250 U, 500 reactions in a final volume of 50 µl	12 032 937 001
	2,500 U, 10 x 250 U, 1,250 reactions in a final volume of 50 µl	12 032 945 001
	5,000 U, 20 x 250 U, 2,500 reactions in a final volume of 50 µl	12 032 953 001
Protector RNase Inhibitor	2,000 U, 40 U/µl	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001

6.4. Trademarks

FASTSTART, MAGNA PURE, TAQMAN, EXPAND and LIGHTCYCLER are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of LifeScience products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country to display country-specific contact information.

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