



# Reverse Transcription & RT-PCR

Reverse transcription, a process that involves a reverse transcriptase (RTase) which uses RNA as the template to make complementary DNA (cDNA), is involved in various applications in molecular biology. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is a powerful tool to detect gene expression, which can assist in diagnosing genetic diseases and detecting cancers. With RT-PCR technology, RNA that is fragmented, degraded, or even in small amounts can be used for downstream applications, including insertions into host-vector systems, construction of cDNA libraries, cloning, sequencing, and characterization of RNA.

In a reverse transcription, a reverse transcriptase (RTase) uses a strand of RNA template to make complementary DNA (cDNA), a process that is the reverse of naturally occurring DNA transcription where RNA is synthesized using DNA templates. An RTase includes two crucial enzymatic components – a DNA polymerase that can operate on both RNA (first strand synthesis) and DNA (second strand synthesis), and a ribonuclease H (RNase H) domain. Each of these domains contributes to the process of cDNA synthesis from RNA.

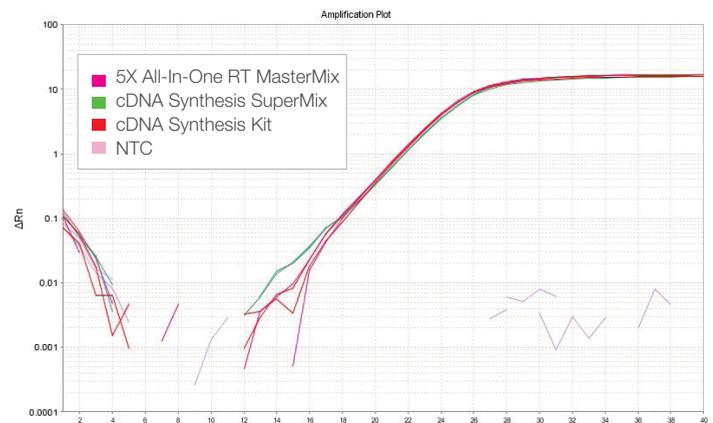
RTases were first isolated from Murine Leukemia Viruses (R-MLV) and Rous Sarcoma Viruses (RSV), retroviruses that use RTases to replicate and incorporate into host genomes. Since then, RTases are used in various applications in molecular biology such as Reverse Transcription Polymerase Chain Reaction (RT-PCR) to study gene expression and to assist in diagnosis of genetic diseases and detection of cancers. With RT-PCR technology, RNA that is fragmented, degraded, or only available in small amounts can still be used for downstream applications.

### 5X All-In-One RT MasterMix

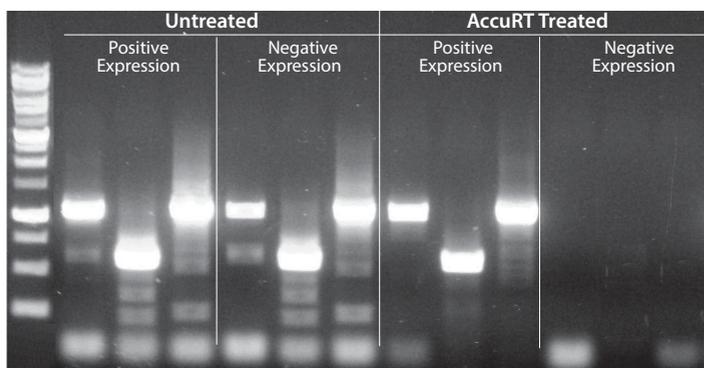
**abm's** 5X All-In-One RT MasterMix enables hassle-free reverse transcription from RNA template to cDNA in just 15 minutes. Optimized for cDNA synthesis over a dynamic range of input RNA, this system eliminates multiple component additions to deliver exceptional reproducibility, precision, and ultimate convenience. For effective removal of contaminating genomic DNA from your samples, pair **abm's** 5X All-In-One RT MasterMix with our AccuRT Genomic DNA Removal Kit. For streamlined RNA extraction directly from mammalian cells, RNA template preparation, and reverse transcription into cDNA, pair **abm's** 5X All-In-One RT MasterMix with our ExCellenCT Lysis Kit.

Our 5X All-In-One RT MasterMixes offer:

- Reduced handling errors due to only one liquid transfer step
- Streamlined protocols suitable for high-throughput applications
- Simple set-ups for any RNA template
- High reproducibility and excellent yields.



**Figure 1: Same reproducibility and comparable cDNA yield from using any of the three RT products abm offers**  
 20 µl RT reactions (100 pg of RNA per reaction) were performed with various **abm's** RT products: cDNA Synthesis Kit, cDNA Synthesis SuperMix, and 5X All-In-One RT MasterMix. 1 µl of each RT product was then used directly as template for GAPDH amplification.



**Figure 2: AccuRT treatment of gDNA contaminated samples and successful removal of gDNA contamination**  
 A positive and negative gene expression analysis samples of RNA (0.1 µg/rxn) contaminated with gDNA (0.8 µg/rxn) were either AccuRT treated or untreated, and were then reverse transcribed in a 20 µl RT reaction. 1 µl of RT product was then used directly in PCR, various primer sets of mTor gene were amplified.

### AccuRT Genomic DNA Removal Kit

The presence of genomic DNA in RNA preparations can cause downstream challenges such as false-positive signals and misrepresentations of gene expression levels. **abm's** AccuRT Genomic DNA Removal Kit eliminates genomic DNA (gDNA) contaminations in RNA samples within 10 minutes—all without loss or degradation of RNA. Combined with **abm's** cDNA synthesis or RT-PCR reagents, the treated gDNA-free RNA is ready for downstream applications such as RT-PCR and qRT-PCR.

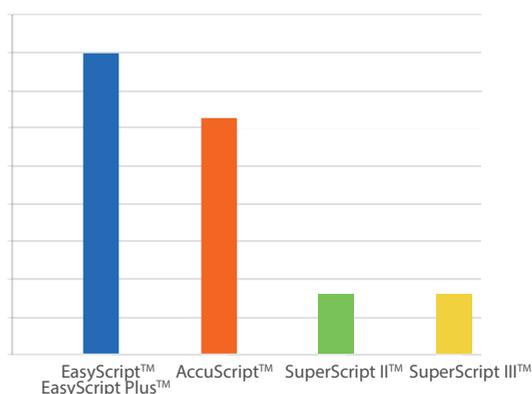
## ExCellenCT Lysis Kit

**abm's** ExCellenCT Lysis Kit is a unique and effective alternative for RNA template extraction and preparation. RNA templates are extracted and processed directly from cultured cells in less than 15 minutes, unveiling RNA/cell lysate ready for reverse transcription without using the hazardous chemicals involved in most RNA extraction and purification protocols. RNA templates generated can then be directly used as template in downstream applications, such as one-step and two step RT-PCR, or real-time PCR systems. In addition, samples can be directly processed in culture wells (96 and 384 wells) for easy handling and high throughput, thus minimizing transfer error and potential sample loss.

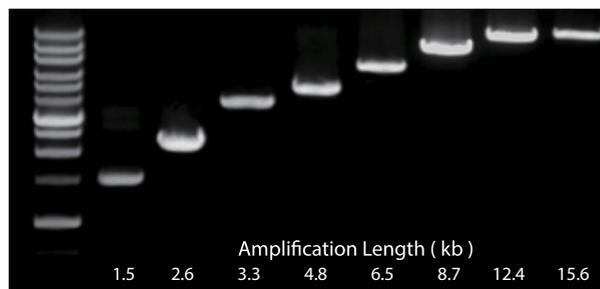
## EasyScript™ and EasyScript Plus™ Reverse Transcriptase

**abm's** EasyScript™ and EasyScript Plus™ Reverse Transcriptases are engineered without intrinsic RNase H activity to increase cDNA synthesis yield and achievable length. EasyScript™ can elongate up to 9 kb while EasyScript Plus™'s extreme processivity enables it to elongate up to 15 kb (Figure 3). EasyScript™ and EasyScript Plus™'s exceptional sensitivity ensure high yields of full length cDNA even with only 0.1 pg of RNA template (Figure 4). Both EasyScript™ and EasyScript Plus™'s unique fidelity-enhancing subunit drastically improves accuracy of reverse transcription, making **abm's** RTases the most reliable on the market (Figure 5).

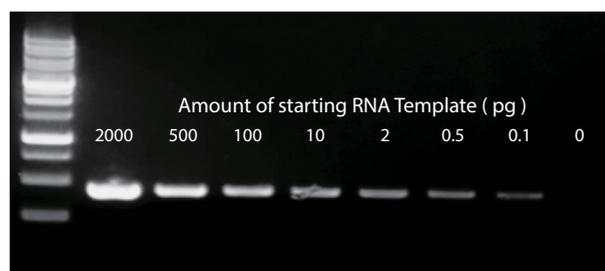
EasyScript™ and EasyScript Plus™ are offered as independent enzymes in optimized buffers, cDNA Synthesis Kits, cDNA Synthesis SuperMixes, and a variety of other formats to suit many applications.



**Figure 5: EasyScript™ RTase series Accuracy Rate**  
EasyScript™ RTases series have the highest accuracy rate compared to leading competitors. Accuracy rate = 1/error rate.



**Figure 3: EasyScript Plus™ Elongation Ability**  
PCR amplification using human cDNA synthesized with EasyScript Plus™, followed by electrophoresis on a 1% agarose gel.



**Figure 4: Sensitivity of EasyScript™ Reverse Transcriptase**  
PCR amplification using human cDNA synthesized with EasyScript™ with varying amounts of starting RNA (2000 pg - 0 pg), followed by electrophoresis on a 1% agarose gel.

## First-Strand cDNA Synthesis Kit

**abm's** cDNA Synthesis Kit contains all reagents needed for generating high quality cDNA and offers the most flexibility in priming methods and reaction optimization. Available in two formats—each employing EasyScript™ and EasyScript Plus™ Reverse Transcriptase for easy selection—the kit offers:

- Maximum flexibility in priming for oligo(dT), random primers, or gene-specific primers
- Robust cDNA synthesis from any RNA template
- High reproducibility and excellent yield.

## First-Strand cDNA Synthesis SuperMix

With minimal effort and maximum ease, **abm's** cDNA Synthesis SuperMix provides highly efficient and specific conversion of RNA to cDNA, even from low abundance transcripts. Available in two formats—each employing EasyScript™ and EasyScript Plus™ Reverse Transcriptase for easy selection—the kit offers:

- Optimal representation of all transcript sequences due to a unique blend of random primers and oligo(dT) primers
- Streamlined protocol for high-throughput applications
- A simple set-up that is easy to use.

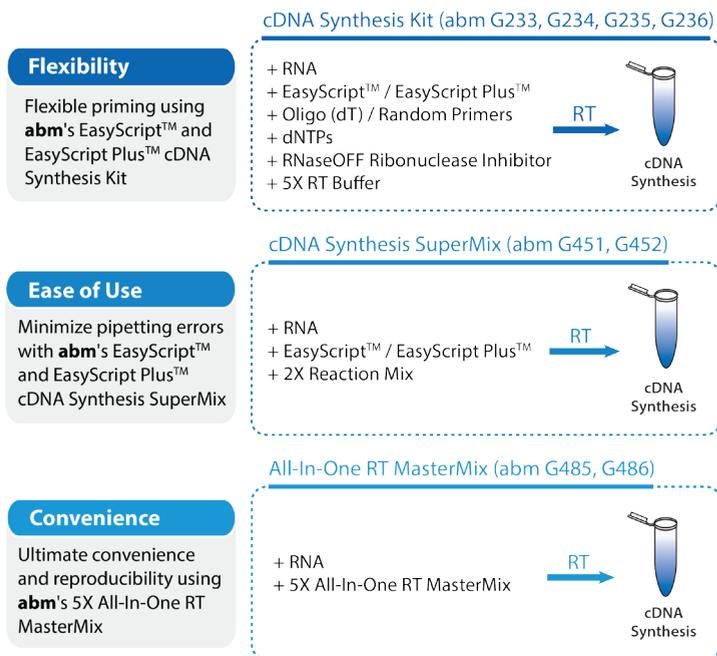


Figure 6: **abm's** Reverse Transcription Kits Selection Guide

### One-Step EvaGreen/TaqProbe qRT-PCR Kit

**abm's** One-Step qRT-PCR Kit combines high-quality enzymes in a proprietary buffer system to deliver precise, accurate, and high-throughput sample analysis that offers the end-user the ultimate convenience. In addition to the complete elimination of primer dimers and a wide selection of reference dyes compatible with a range of qPCR machines, this kit also offers:

- Improved fidelity and yield for reverse transcription
- Prevention of template RNA degradation with RNaseOFF Ribonuclease Inhibitor
- Superb sensitivity and signal-to-noise ratio
- Significant reduction in non-specific PCR amplification by utilizing HotStart Taq DNA polymerase
- Fully optimized low-copy gene detection with high-quality, full-length cDNA from as little as 0.01 pg of RNA
- Reduced contamination risk with streamlined single tube reaction protocol.

### ExCellenCT One-Step EvaGreen/TaqProbe qRT-PCR Kit

**abm's** ExCellenCT Lysis Kit is the alternative method of choice for extraction and processing of RNA templates directly from cells in only 15 minutes. **abm's** proprietary qRT-PCR Enzyme Mix contains stabilizers and enhancers that optimize RT and PCR reactions in a real-time "single step". Coupled together, this complete system provides the ultimate convenience in generating consistent, reproducible, and accurate results directly from 10-10<sup>5</sup> of cultured cells.

### miRNA cDNA Synthesis Kit

**abm's** miRNA cDNA Synthesis Kit is a complete system for the efficient synthesis of first strand miRNA from total RNA templates. The kit also comes with an option of the Poly(A) Tailing step as well as a unique miRNA Oligo d(T) adapter that anneals to the Poly(A) tail of miRNA template, thus facilitating easy cDNA synthesis. The first strand of miRNA's cDNA can be directly used as a template for the qPCR-based analysis of miRNA expression in a given sample using **abm's** EvaGreen miRNA qPCR MasterMixes.

### Two-Step RT-PCR Kit

**abm's** Two-Step RT-PCR Kit contains our EasyScript cDNA Synthesis SuperMix for optimal RNA to cDNA conversion, and our TaqPlus 2X PCR MasterMix with dye that provides all ingredients for PCR. Since RNA is unstable, a pool of the more stable cDNA can be stored separately in the "two-step" protocol to be used as the template in a series of subsequent PCR reactions. With a simple and streamlined protocol, **abm's** Two-Step RT-PCR Kit provides excellent cDNA yield and robust PCR performance.

### One-Step RT-PCR Kit

**abm's** One-Step RT-PCR Kit simplifies reverse transcription and subsequent PCR into a single tube reaction, eliminating the buffer changing steps which may cause contamination. Offering extreme sensitivity, specificity, and high product yield, the "one-step" method is an efficient and economic alternative to the conventional "two-step" RT-PCR approach. Requiring the use of gene-specific primers, it is more suitable when a known gene is being targeted for study.

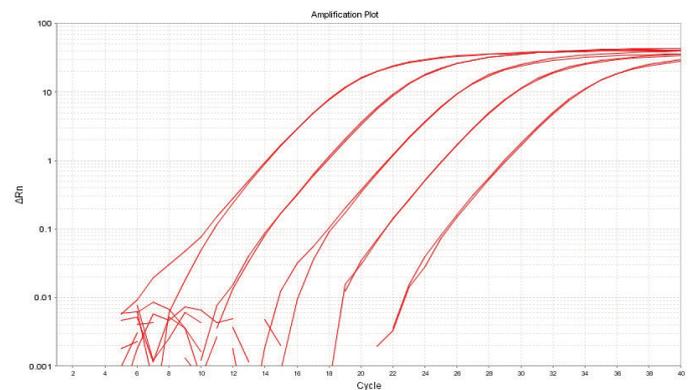


Figure 7: 20 µl One-Step qRT-PCR reactions (20 ng, 2 ng, 200 pg, 2 pg or 2 pg of RNA per reaction) were carried out with **abm** Step EvaGreen qRT-PCR kit for GAPDH amplification.

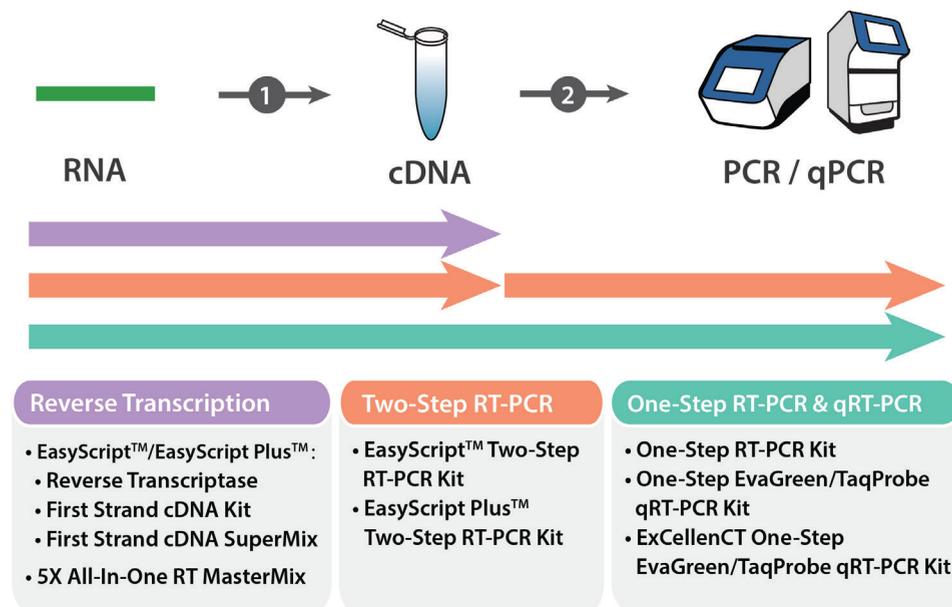


Figure 8: abm's Reverse Transcription &amp; RT-PCR Products

## Reverse Transcription & RT-PCR Product List

Cat. No.	Product Name	Size
G485, G486, G490	5X All-In-One RT MasterMix	25/100/200 reactions
G492	5X All-In-One RT MasterMix with AccuRT Genomic DNA Removal Kit	100 reactions
G916	5X All-In-One RT MasterMix with ExCellenCT Lysis Kit	100 reactions
G231, G232	EasyScript™ Reverse Transcriptase (PCR)	25/100 reactions
G177, G237	EasyScript Plus™ Reverse Transcriptase (PCR)	25/100 reactions
G233, G234	EasyScript™ cDNA Synthesis Kit	25/100 reactions
G491	EasyScript™ cDNA Synthesis Kit with AccuRT Genomic DNA Removal Kit	100 reactions
G235, G236	EasyScript Plus™ cDNA Synthesis Kit	25/100 reactions
G904, G905	Total-Transcriptome cDNA Synthesis Kit	25/100 reactions
G451, G452	EasyScript™ cDNA Synthesis SuperMix	25/100 reactions
G453, G454	EasyScript Plus™ cDNA Synthesis SuperMix	25/100 reactions
G281, G282	EasyScript™ Two-Step RT-PCR Kit	25/100 reactions
G283, G284	EasyScript Plus™ Two-Step RT-PCR Kit	25/100 reactions
G174, G174-dye	One-Step RT-PCR Kit (with Dye)	100 reactions
G471-R, LR, iC, S	One-Step EvaGreen qRT-PCR	100 reactions
G917-R, LR, iC, S	ExCellenCT One-Step EvaGreen qRT-PCR	25 preps, 100 reactions
G493-P, PL, PC, PS	One-Step TaqProbe qRT-PCR	100 reactions
G918-P, PL, PC, PS	ExCellenCT One-Step TaqProbe qRT-PCR	25 preps, 100 reactions
G269, G270	miRNA cDNA Synthesis Kit	25/100 reactions
G902, G903	miRNA cDNA Synthesis Kit, with Poly(A) Polymerase Tailing	25/100 reactions

## More Resources

For more information about Reverse Transcription & RT-PCR, visit our Knowledge Base and YouTube Channel!

### Knowledge Base

[https://www.abmgood.com/marketing/knowledge\\_base.php](https://www.abmgood.com/marketing/knowledge_base.php)

### YouTube Channel

[www.youtube.com/c/abmgood](http://www.youtube.com/c/abmgood)

### *Polymerase Chain Reaction - An Introduction*

<https://youtu.be/matsiHSuoOw>

### *Polymerase Chain Reaction - Variations of DNA Polymerase*

<https://youtu.be/oqeV72oYfD0>

### *RT Products Overview*

[https://youtu.be/vlv4-24Gc\\_E](https://youtu.be/vlv4-24Gc_E)

