HotStart-IT®
SYBR® Green
One-Step qRT-PCR
Master Mix Kit

Product Number 75770

STORAGE
Store at -15°C to -30°C.

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.
COMPONENTS
All reagents have been extensively tested and carefully prepared to meet USB® standards. USB recommends the reagents be used as directed in order to achieve the best possible results.

The following components are included with each kit:

<table>
<thead>
<tr>
<th>Components</th>
<th>100 reaction kit</th>
<th>500 reaction kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>HotStart-IT® SYBR® Green qPCR Master Mix (2X), PN 75762</td>
<td>2 x 1.25 ml</td>
<td>1 x 12.5 ml</td>
</tr>
<tr>
<td>25mM MgCl₂, PN 71167</td>
<td>1 x 1 ml</td>
<td>5 x 1 ml</td>
</tr>
<tr>
<td>ROX™ Passive Reference Dye, PN 75768</td>
<td>1 x 100 µl</td>
<td>1 x 500 µl</td>
</tr>
<tr>
<td>Fluorescein Passive Reference Dye, PN 75767</td>
<td>1 x 100 µl</td>
<td>1 x 500 µl</td>
</tr>
<tr>
<td>M-MLV RT, PN 75783</td>
<td>1 x 40 µl</td>
<td>1 x 200 µl</td>
</tr>
<tr>
<td>RNase Inhibitor (10 units/µl), PN 75782</td>
<td>1 x 40 µl</td>
<td>1 x 200 µl</td>
</tr>
<tr>
<td>RNase-Free Water, DEPC-Treated, PN 70783</td>
<td>3 x 1 ml</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>

HotStart-IT® SYBR® Green qPCR Master Mix (2X), PN 75762: This Master Mix is a 2X pre-mixed formulation containing HotStart-IT® Taq DNA Polymerase, MgCl₂, Ultrapure nucleotides, and SYBR Green I in an optimized reaction buffer for use in real-time, quantitative PCR reactions. Magnesium and nucleotide concentrations are at 5mM and 0.4mM, respectively. There is no dUTP in the mix so carry-over contamination prevention using UDG cannot be performed.

The enclosed reagents should be stored at -15°C to -30°C (NOT in a frost-free freezer). After thawing for use, keep reagents on ice. Light sensitive components should be protected from excessive light exposure.
QUALITY CONTROL

HotStart-IT® SYBR® Green One-Step qRT-PCR Master Mix Kit is a Tested User Friendly™ product, assuring reliable results. Release specifications for the kit are based on the following functional assays.

1) Real-Time Assay: Real-time qRT-PCR reactions were performed on an ABI 7500 Fast Instrument using primers specific to a 122 bp region of the human GAPDH gene and human total RNA as template. Product specifications require that the correlation coefficient from a linear regression over five orders of magnitude (10 pg to 100 ng) must be greater than or equal to 0.95.

2) Polymerase Blocking Assay: The assay compared the polymerase activity of the HotStart-IT SYBR Green qPCR Master Mix relative to Taq PCR Master Mix without hot start. The reaction mixture contained 2 pmol of overlapping, extendable oligonucleotides in a 25 µl reaction volume. Following incubation at 25°C for 4 hours, the HotStart-IT SYBR Green qPCR Master Mix blocked at least 90% of the activity relative to the master mix without hot start capability. All components were tested for contaminating ssDNA and dsDNA endonucleases, ssDNA and dsDNA exonucleases, and ribonucleases.

SAFETY WARNINGS AND PRECAUTIONS

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: All chemicals should be considered as potentially hazardous. We, therefore, recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coat, safety glasses, and gloves. Care should be taken to avoid contact with skin and eyes. In the case of contact with skin or eyes, wash immediately with water. See MSDS (Material Safety Data Sheet) for specific advice.

DESCRIPTION

HotStart-IT® SYBR® Green One-Step qRT-PCR Master Mix Kit provides maximum convenience and optimal performance for real-time, quantitative analysis of RNA templates in a single reaction format. The RT-PCR process converts and amplifies single-stranded RNA template yielding double-stranded DNA product. One-Step RT-PCR uses gene specific primers, designed to match RNA/cDNA targets, in a single-tube, one-step reaction. This approach offers tremendous convenience when applied to analysis of single targets from multiple RNA samples. Also, it minimizes the possibility of introducing contaminants into reactions between the RT and PCR steps, since both steps are carried out sequentially without opening the reaction tubes between the steps.

HotStart-IT SYBR Green One-Step qRT-PCR Master Mix Kit is supplied with separate tubes of M-MLV RT and RNase Inhibitor and a 2X Master Mix containing HotStart-IT Taq DNA Polymerase, MgCl₂, Ultrapure nucleotides, and SYBR Green I in an optimized reaction buffer. HotStart-IT SYBR Green qPCR Master Mix uses a novel hot start method designed and developed at USB called primer sequestration. With this method, the HotStart-IT protein binds and sequesters primers at lower temperatures making them unavailable for use by Taq DNA Polymerase. Following reverse transcription and the subsequent heat denaturation step, the primer binding protein is inactivated and the primers are released. This novel hot start feature increases the specificity and sensitivity of SYBR-based qRT-PCR reactions by reducing primer-dimer formation.

SYBR Green I Dye is used in this kit to detect any double-stranded DNA that accumulates during the amplification process and also allows melt-curve analyses. No fluorescent probes are required. This kit exhibits excellent sensitivity as it can detect fewer than 10 target copies, performs over a broad, linear dynamic range of 6 to 7 orders of magnitude, and is compatible with most real-time PCR instruments. Individual kit components have been carefully formulated to obtain optimal activity of M-MLV RT, Taq DNA Polymerase, and SYBR Green I to allow highly sensitive and specific detection of RNA transcripts from either total RNA or poly(A)⁺ mRNA. Separate tubes of the passive reference dyes ROX™ (for ABI and Stratagene instruments) and fluorescein (for Bio-Rad instruments) are included for added convenience to allow normalization of well-to-well variations.
MATERIALS NOT SUPPLIED

Necessary reagents:

**RNA template:** Total RNA and poly(A)+ mRNA can be prepared by standard purification methods such as the PrepEase® RNA Spin Kit (PN 78766) and PrepEase® Plant RNA Spin Kit (PN 78771), Oligo-p(dT) Cellulose (PN 71547), acid-guanidinium thiocyanate-phenol-chloroform extraction (6-7) or TRIzol® procedures. RNA may also be obtained from commercial suppliers. RNA should be highly purified and free of RNase, polysaccharide, and proteoglycan contamination (7). Ideally, RNA should also be free of DNA contamination.

**Oligonucleotide primers:** Oligonucleotide primers can be designed according to standard methods (8). Longer oligonucleotides (> 25 bases) and those with higher melting temperatures (> 60°C) are recommended to achieve more specific amplification. Gene-specific primers which flank an intron or cross an exon-exon border are useful as a control to distinguish amplification from RNA versus contaminating DNA.

Optional reagents:

**Enhancing additives:** Solvents such as dimethyl sulfoxide (DMSO), glycerol, trehalose, and betaine can improve results for qRT-PCR on certain targets with a high degree of secondary structure (9-12).

Necessary equipment:

**Liquid handling supplies** such as PCR-grade thin-walled tubes or plates, pipettes, pipettors, and a centrifuge are required. Use plastic tubes, plates, and pipette tips that are certified RNase-free, in order to prevent RNase contamination of samples. Also, the use of barrier-tip pipettes and dedicated PCR pipettors are strongly recommended in order to avoid RNase contamination.

**Latex gloves (powder-free)** should be used for handling reagents and equipment in order to decrease the probability of introducing RNases into samples.

**Real-time thermal cycler** for incubations between 4°C and 95°C, as well as fluorescent detection and quantification.

**Equipment** such as a standard horizontal gel apparatus and a UV transilluminator or fluorescence image scanner can also be used for analysis of qRT-PCR products.

PROTOCOL

This protocol applies to a single reaction where M-MLV RT, RNase Inhibitor, RNA template, primers, and water are added to the HotStart-IT® SYBR® Green qPCR Master Mix. For multiple reactions, increase the volumes of the reaction components proportionally.

1. Thaw the master mix and other necessary frozen reagents at room temperature. Mix thoroughly, briefly spin to collect tube contents and then place on ice. The M-MLV RT and RNA samples should always be kept on ice.

2. Assemble reaction tubes or plates on ice.

3. This table shows recommended component volumes. It is highly recommended to make a master mix for at least 10 reactions to reduce pipetting errors.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume for 50 µl reaction</th>
<th>Volume for 20 µl reaction</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HotStart-IT® SYBR® Green qPCR Master Mix (2X)</td>
<td>25 µl</td>
<td>10 µl</td>
<td>1X (has 2.5mM MgCl₂)</td>
</tr>
<tr>
<td>M-MLV RT</td>
<td>0.4 µl</td>
<td>0.16 µl</td>
<td>1X</td>
</tr>
<tr>
<td>RNase Inhibitor</td>
<td>0.4 µl</td>
<td>0.16 µl</td>
<td>1X</td>
</tr>
<tr>
<td>10µM Forward Primer</td>
<td>0.5-5.0 µl</td>
<td>0.2-2.0 µl</td>
<td>0.1-1.0µM*</td>
</tr>
<tr>
<td>10µM Reverse Primer</td>
<td>0.5-5.0 µl</td>
<td>0.2-2.0 µl</td>
<td>0.1-1.0µM*</td>
</tr>
<tr>
<td>Template RNA</td>
<td>X µl</td>
<td>X µl</td>
<td>as needed, &lt;1 µg</td>
</tr>
<tr>
<td>Passive Reference Dye (optional: ROX for ABI and Stratagene; Fluorescein for Bio-Rad)</td>
<td></td>
<td></td>
<td>see included Passive Reference Dye protocols for details</td>
</tr>
<tr>
<td>RNase-Free Water, DEPC-Treated</td>
<td>up to 50 µl</td>
<td>up to 20 µl</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Optimal primer concentration is 0.2µM. In order to avoid primer-dimers and non-specific products, use ≤ 0.5µM. Because the reverse primer is also used during the initial reverse transcription step, it may be helpful to double the amount of reverse primer only.

†Total RNA may be used at 1 pg to 100 ng and poly(A)+ mRNA may be used at 100 fg to 100 ng per reaction.
NOTE: It is useful to test for contaminating genomic DNA in the RNA sample(s) by performing a control reaction which omits the M-MLV RT. Since there is no reverse transcriptase in this reaction, any observed product must have been generated from a DNA source. Also, to confirm the absence of general DNA or RNA contamination in other reagents, perform a No-Template-Control reaction.

4. Optional: If optimizing Mg²⁺ concentration, add 2.0 µl of 25mM MgCl₂ per 50 µl reaction for each additional 1mM Mg²⁺ required. Subtract this volume from the amount of water needed.

5. Cap tubes or seal plates with optically clear caps or film. Mix tubes or plates by gentle vortexing and then spin to collect contents without bubbles (e.g. 2-5 min at 1000-2000 x g).

6. The following tables show recommended cycling conditions:

### Combined Annealing/Extension Step Cycling Program

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cycle of</td>
<td>50°C for 10 min</td>
<td>Reverse transcription of RNA by M-MLV RT to generate first-strand cDNA.</td>
</tr>
<tr>
<td>1 cycle of</td>
<td>95°C for 2 min</td>
<td>HotStart-IT Binding Protein and M-MLV RT inactivation.</td>
</tr>
<tr>
<td>35-45 cycles of</td>
<td>95°C for 15 sec</td>
<td>60°C for 30-60 sec: Acquire real-time fluorescence data during this step.</td>
</tr>
</tbody>
</table>

Melt-Curve Analysis: Recommended to distinguish specific products from non-specific ones. Consult the thermal-cycler manual for details.

### Fast Cycling Program (e.g. ABI 7500 in Fast Mode)

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cycle of</td>
<td>50°C for 5 min</td>
<td>Reverse transcription of RNA by M-MLV RT to generate first-strand cDNA.</td>
</tr>
<tr>
<td>1 cycle of</td>
<td>95°C for 2 min</td>
<td>HotStart-IT Binding Protein and M-MLV RT inactivation.</td>
</tr>
<tr>
<td>35-45 cycles of</td>
<td>95°C for 3-5 sec</td>
<td>60°C for 15-30 sec: Acquire real-time fluorescence data during this step.</td>
</tr>
</tbody>
</table>

Melt-Curve Analysis: Recommended to distinguish specific products from non-specific ones. Consult the thermal-cycler manual for details.

7. If desired, confirm that specific RT-PCR products have been generated by agarose gel electrophoresis. Amplicons may be detected on gels with ethidium bromide or without ethidium bromide using the SYBR Green I (included in the reaction mix).
**SUPPLEMENTARY INFORMATION**

**RNA Quality**

The quality of the RNA sample is the most important factor affecting the outcome of qRT-PCR reactions. Quality can be defined in terms of both purity and integrity (i.e. proportion of the RNA sample which is full-length). Many purification methods can be used to prepare RNA, provided the methods yield RNA which is essentially free of contaminating DNA, proteins, polysaccharides or proteoglycans, phenol, ethanol, and salts. These contaminants inhibit the activity of M-MLV Reverse Transcriptase, which reduces qRT-PCR amplification yields\(^7\). After RNA purification with protocols that use solvents such as phenol or guanidinium salts, we strongly recommend washing the RNA pellet at least twice with cold 70-75% ethanol prior to dissolving the RNA pellet in DEPC-treated solutions to help remove any traces of these solvents.

The purity of the RNA sample should be estimated by measuring its absorbance at 260 and 280 nm in TE buffer. \(\text{A}_{260}/\text{A}_{280}\) ratios should fall between 1.7-2.1. If ratios fall outside this range, re-precipitation of the RNA or column purification may be necessary.

The integrity of the RNA may be assessed by performing denaturing agarose gel electrophoresis. High-quality, full-length RNA will exhibit two dominant and discrete bands, composed of 28S and 18S ribosomal RNA. These rRNA bands should have minimal smearing below them, indicating absence of degradation. RNA of the highest quality has a 28S rRNA band which stains about twice as intense as the 18S rRNA band. Maintain RNA integrity by storing samples in TE (10mM Tris-HCl, pH 7, 1mM EDTA), 0.1mM EDTA, or DEPC-Treated Water at -80°C.

Reduce or eliminate any suspected genomic DNA contamination by treating the RNA sample with USB recombinant DNase I (PN 78311). This is particularly important if experiments generate targets of the same size from RNA and contaminating genomic DNA (i.e. no intron present in genomic DNA target).

**Amount of RNA per Reaction**

Total RNA may be used at 1 pg to 100 ng and poly(A)\(^+\) mRNA may be used at 100 fg to 100 ng per reaction. Greater representation of the specific target within a population of DNA molecules allows the use of lower amounts of RNA. Since poly(A)\(^+\) mRNA comprises approximately 1 to 5% of the total RNA\(^13\), a specific target will be more abundant in poly(A)\(^+\) mRNA than in total RNA. For best results, when working with dilute stocks of RNA (less than 100 ng/µl), freshly prepare the dilute stocks from concentrated stocks rather than subjecting dilute stocks to multiple freeze-thaw cycles.

**RNase Contamination**

In order to avoid RNase contamination, maintain a clean, dust-free work area, use powder-free latex gloves, dedicate a set of pipettors and a microcentrifuge specifically for RNA work, use certified RNase-free barrier tips and plastic tubes, and store RNA in concentrated solutions (~1 µg/µl) at -80°C\(^14\). Ideally, use solutions that are certified RNase-free or that have been DEPC-treated to inactivate any RNases present. Keep RNA samples on ice while setting up the experiment. Also, it is highly recommended to use the RNase Inhibitor provided in the kit to reduce the effect of RNases. Avoiding potential sources of RNase contamination (ungloved hands, contaminated pipettors, etc.) will help to prevent sample degradation.

**Primer Design**

During One-Step qRT-PCR, two gene specific primers are designed per qRT-PCR product. The 3’ or reverse primer is complementary to the target RNA. The 5’ or forward primer is complementary to the corresponding first-strand of cDNA. In reverse transcription, the reverse primer initiates synthesis of the first-strand of cDNA by M-MLV RT. In PCR, the forward primer initiates synthesis of the second strand of cDNA during the first PCR cycle by Taq DNA Polymerase. Taq then uses both primers to exponentially amplify the two DNA strands during subsequent PCR cycles. The resulting PCR product is detected and quantified in real-time by using the dsDNA-specific binding dye, SYBR\(^\text{®}\) Green I.
General rules for designing primers can be found in many texts. In general, primers should range in length from 18 to 30 nucleotides, exhibit G+C content similar to each other (ideally in the range of 40 to 60%), and exhibit $T_m$ values ranging from 55 to 65°C that are closely matched to each other. $T_m$ values may be estimated using the following equation: $T_m(°C) = 2(A+T) + 4(G+C)$. More accurate methods for calculation of $T_m$ values may also be applied.

When designing qRT-PCR primers, make sure the size of the expected amplicon is between 80-200 bp, with an optimal length of 100-150 bp. Primers that do not fit these criteria may also function well, but empirical testing is required. Using computer programs designed to select appropriate primers for qRT-PCR in a given sequence is highly recommended. In addition, several public primer databases are available on the internet. These databases contain gene-specific primer sets that have been validated for gene expression analysis by real-time PCR. Some examples of databases include PrimerBank (http://pga.mgh.harvard.edu/primerbank/) and RTPrimerDB (http://medgen.ugent.be/rtprimerdb/). Other resources for PCR primers, oligos databases and design tools can be found at http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos.

When using a set of primers for the first time, we recommend validating this set first by running a small amount of the reaction (e.g. 5 µl) on an agarose gel to confirm the presence of a single product and second, by analyzing the dissociation or melting curve which should generate a single peak.

It is useful to design primer sets that give different amplification products from messenger RNA than from genomic DNA that may be a sample contaminant. Whenever possible, primers should flank an intron or span an exon-exon border. For primers flanking an intron, the PCR product will be smaller from RNA compared to that from contaminating genomic DNA. For primers crossing an exon-exon border, PCR product should not be generated from genomic DNA. Be aware that common housekeeping genes such as β-actin or GAPDH have intron-less pseudogenes in many organisms. In those cases, it is important to have RNA which is completely free of contaminating genomic DNA.

Thermal Cycler Program Design

One-Step qRT-PCR uses a single thermal cycler program for reverse transcription, inactivation of reverse transcriptase, and real-time, quantitative detection of the PCR products. This kit is compatible with most real-time PCR instruments and the cycling programs given in the Protocol section are a good starting point for program design. Several adjustments may improve results as the optimal cycling conditions will vary from one instrument to another. Consult the specific qPCR instrument manual for advice and suggestions on the best possible protocol.

Optimizing qRT-PCR

The sensitivity and yield of qRT-PCR reactions can be improved by increasing the primer concentrations to above 0.5µM. There is occasionally some loss of specificity when adding more primers, but this is often overcome by increasing the annealing temperature by increments of approximately 2°C. If specificity is preferred rather than sensitivity, decrease the concentration of primers in the qRT-PCR reaction to between 0.1-0.2µM. It may also be necessary to design multiple primer sets for each target, at varying distances along the transcript, to obtain the best possible results. It is highly recommended to use the suggested RT reaction temperature of 50°C which helps melt RNA secondary structure and provides for better first-strand cDNA synthesis.

Suggestions for Difficult Templates

Successful amplification with the HotStart-IT SYBR Green One-Step qRT-PCR Master Mix Kit often requires little or no optimization. If targets have a high-degree of secondary structure and/or high G+C content (e.g. > 60%), adding certain supplements, such as DMSO, glycerol, trehalose, and/or betaine to the qRT-PCR reaction may improve results. DMSO and glycerol may be added at final concentrations ranging from 1 to 10% (v/v). Trehalose may be added to 0.6M final concentration. Betaine (5M stock, PN 77507) may be added at 0.5M to 2.0M final concentration. Betaine and trehalose have been reported to thermostabilize proteins in general. Thus, the RT reaction temperature may be elevated in their presence, possibly melting secondary structure. All of these solvents tend to decrease $T_m$ values for double-stranded DNA, thus its presence in reactions may result in a decrease in the optimum annealing temperature by several degrees.

Addition of supplemental magnesium chloride may also improve results. Benefits are usually observed within a narrow range and final Mg$^{2+}$ concentrations above 6mM should be avoided.
**TROUBLESHOOTING**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes and solutions</th>
</tr>
</thead>
</table>
| No amplification product, weak fluorescent signal, or poor sensitivity | 1. Use more RNA template, between 1 ng to 100 ng of total RNA or 100 pg to 100 ng of poly(A)+ mRNA. Verify the integrity of the RNA by gel electrophoresis. Confirm concentration of the RNA with a spectrophotometer. If the RNA has been damaged or degraded, prepare a fresh stock.  
2. Remove possible RT inhibitors such as SDS, EDTA, salts, etc. from the RNA sample by two 70-75% ethanol washes, re-precipitation, or column purification.  
3. Increase the number of PCR cycles, up to 45 cycles, for low-copy targets.  
4. Increase the concentration of primers, for example, from 0.2µM to 0.8µM.  
5. Double the concentration of the reverse primer relative to the forward primer.  
6. Increase the magnesium final concentration in 0.5mM increments.  
7. Test a range of PCR annealing temperatures. Start with an annealing temperature 5-10°C below the primer(s) Tm and increase in 1-2°C increments. Annealing temperatures that are either too high or too low can result in absence of product.  
8. Use a RT temperature of 50°C to help melt RNA secondary structure.  
9. If template and/or primers exhibit G+C content greater than ~60%, supplement reactions with additives suggested in the Supplementary Information section. In general, the optimal amount of additives for a given primer/template combination needs to be determined empirically.  
10. Mix the qRT-PCR Master Mix and reactions well. Spin down contents to the bottom of the tube.  
11. Design new primers. Use an oligonucleotide design computer program such as those listed in the Supplementary Information section. |

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes and solutions</th>
</tr>
</thead>
</table>
| Multiple peaks on melt-curve analysis, higher than expected fluorescence, or non-specific bands following electrophoresis | 1. Use less RNA template.  
2. Reduce the number of PCR cycles.  
3. Raise the annealing temperature in 1-2°C increments.  
4. Decrease the concentration of primers, for example from 0.4µM to 0.1µM. Also, never use oligo (dT) or random primers in One-Step qRT-PCR reactions.  
5. Check for contaminating genomic DNA by omitting the M-MLV RT from the reactions. If products of expected size are still generated, pre-treat RNA samples with RNase-free DNase I (PN 78311). Also, design primers which flank an intron or span an exon-exon border.  
6. If products are generated from a No-Template-Control reaction, completely clean the work area and pipettors and replace any solutions or stocks that can be replaced. Maintain a meticulous RNase-free environment using the suggestions given in the Supplementary Information section.  
7. Supplement the reactions with additives that improve amplification of G+C rich templates as suggested in the Supplementary Information section.  
8. Check the integrity of the RNA stocks by gel electrophoresis. Prepare fresh RNA stocks or fresh dilutions if necessary.  
9. Design new primers. Use an oligonucleotide design computer program such as those listed in the Supplementary Information section. |

If problems persist please contact USB Technical Support for assistance at (800) 321-9322 or techsupport@usbweb.com. Additional information such as new product listings, updated protocols and TechTips, may be found at our website, www.usbweb.com. For technical support outside the U.S., please visit our website for up-to-date contact information on the USB product distributor within your area.
### RELATED PRODUCTS

#### Reverse Transcription Enzymes and RT-PCR Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>HotStart-IT® Probe One-Step qRT-PCR Master Mix Kit</td>
<td>Real-time quantitative analysis of RNA templates in a single reaction format</td>
<td>100 reactions 500 reactions</td>
<td>75772</td>
</tr>
<tr>
<td>First-Strand cDNA Synthesis Kit for Real-Time PCR</td>
<td>Conversion of RNA into first-strand cDNA for real-time PCR</td>
<td>50 reactions</td>
<td>75780</td>
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<tr>
<td>M-MLV Reverse Transcriptase</td>
<td>cDNA synthesis, low RNase H activity</td>
<td>25,000 units 100,000 units</td>
<td>78306</td>
</tr>
<tr>
<td>AMV Reverse Transcriptase</td>
<td>cDNA synthesis</td>
<td>200 units 1,000 units</td>
<td>78311 70041Y 70041Z</td>
</tr>
<tr>
<td>Ribonuclease Inhibitor, Recombinant (40 units/µl)</td>
<td>Protect RNA</td>
<td>5,000 units</td>
<td>71571</td>
</tr>
<tr>
<td>Oligo dT(12-18) Primer</td>
<td>Priming synthesis of cDNA from mRNA</td>
<td>100 µl (2.5 nmol)</td>
<td>77405</td>
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<tr>
<td>One-Step RT-PCR Kit</td>
<td>RT-PCR, analysis of multiple templates</td>
<td>50 reactions</td>
<td>78350</td>
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<tr>
<td>Two-Step RT-PCR Kit</td>
<td>RT-PCR, analysis of multiple genes</td>
<td>50 RT/100 PCR reactions</td>
<td>78355</td>
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<tr>
<td>RT Script Kit</td>
<td>RT synthesis of cDNA for cloning, arrays, and RT-PCR</td>
<td>50 reactions</td>
<td>78360</td>
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<td>RNase I, Recombinant, RNase-Free</td>
<td>Removal of DNA prior to RT-PCR</td>
<td>1,000 units 2,500 units</td>
<td>78311</td>
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</tbody>
</table>

#### PCR Enzymes and Related Products

<table>
<thead>
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<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq DNA Polymerase</td>
<td>PCR</td>
<td>50 units 250 units 1,000 units 5 × 250 units 5,000 units</td>
<td>71160</td>
</tr>
<tr>
<td>FideliTaq™ DNA Polymerase</td>
<td>PCR</td>
<td>50 units 250 units 1,000 units 5 × 250 units 5,000 units</td>
<td>71180</td>
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<tr>
<td>Taq PCR Kit</td>
<td>PCR, including all necessary reagents</td>
<td>100 reactions</td>
<td>71161</td>
</tr>
<tr>
<td>Taq PCR Master Mix (2X)</td>
<td>PCR reaction mix (2X), ready-to-use</td>
<td>100 reactions (125 units)</td>
<td>71162</td>
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<tr>
<td>FideliTaq PCR Master Mix (2X)</td>
<td>PCR reaction mix (2X), ready-to-use</td>
<td>100 reactions</td>
<td>71182</td>
</tr>
<tr>
<td>FideliTaq PCR Master Mix Plus</td>
<td>PCR reaction mix, ready-to-use</td>
<td>100 reactions</td>
<td>71183</td>
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<tr>
<td>ExoSAP-IT®</td>
<td>Clean-up of PCR products</td>
<td>20 reactions 100 reactions 500 reactions 2,000 reactions 5,000 reactions</td>
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### REFERENCES

### Ultrapure Nucleotides

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<th>Application</th>
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<tbody>
<tr>
<td>PCR Nucleotide Mix, 10mM each of dATP, dCTP, dGTP, and dTTP</td>
<td>RT and/or PCR, nucleotides</td>
<td>500 µl</td>
<td>77212</td>
</tr>
<tr>
<td>PCR Nucleotide Mix, 25mM each of dATP, dCTP, dGTP, and dTTP (Set of Four)</td>
<td>RT and/or PCR, nucleotides</td>
<td>4 × 25 µmol (250 µl)</td>
<td>77100</td>
</tr>
</tbody>
</table>

### Additives for PCR

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Chloride, 25mM Solution</td>
<td>Supplement for RT and/or PCR</td>
<td>1 ml</td>
<td>71167</td>
</tr>
<tr>
<td>Glycerol, Nuclease-Free, Ultrapure</td>
<td>Supplement for RT and/or PCR</td>
<td>500 ml</td>
<td>16374</td>
</tr>
<tr>
<td>Betaine, 5M Solution, Ultrapure</td>
<td>Supplement for RT and/or PCR</td>
<td>1.5 ml</td>
<td>77507</td>
</tr>
<tr>
<td>α,α-Trehalose, Dihydrate</td>
<td>Supplement for RT and/or PCR</td>
<td>10 mg</td>
<td>22515</td>
</tr>
<tr>
<td>BSA, 50 mg/ml Solution, Non-Acetylated, Ultrapure</td>
<td>Supplement for RT and/or PCR</td>
<td>50 mg</td>
<td>10921</td>
</tr>
</tbody>
</table>

### Ultrapure RNA Reagents

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl Pyrocarbonate (DEPC)</td>
<td>RNase inactivation</td>
<td>25 ml</td>
<td>14710</td>
</tr>
<tr>
<td>RNA Solutions Kit</td>
<td>Storage and handling of RNA</td>
<td>8 × 100 ml per pk, 1 kit</td>
<td>75903</td>
</tr>
<tr>
<td>TE Buffer (1X)</td>
<td>Storage of RNA/DNA</td>
<td>10 × 1 ml</td>
<td>75893</td>
</tr>
<tr>
<td>Water, RNase-Free</td>
<td></td>
<td>10 × 1 ml</td>
<td>71783</td>
</tr>
</tbody>
</table>

### Ultrapure Electrophoresis Reagents

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, RNase-Free, DEPC-Treated</td>
<td>Gel electrophoresis</td>
<td>25 ml</td>
<td>70783</td>
</tr>
</tbody>
</table>

### USB CORPORATION

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USB products distributed outside the USA:
Please visit the USB website at www.usbweb.com for up-to-date contact information within your area.
Material Safety Data Sheet
Revision: 11/15/2007

IDENTIFICATION OF THE \nPRODUCT NAME: HotStart-IT SYBR® Green 75770 None
SUBSTANCE/PREPARATION AND COMPANY
SUPPLIER: USB Corporation
EMERGENCY CONTACT: Chemtrec: (800) 424-9300
26111 Miles Road, Cleveland, Ohio 44128 Phone: (216) 765-5000
Outside USA & Canada: 703-527-3887
Please visit our website at www.usbweb.com for contact information on USB product distributors within your area.

HAZARDOUS COMPONENTS

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>CAS NO.</th>
<th>% WT</th>
<th>TLV</th>
<th>CHIP R &amp; P Phrases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>56-81-5</td>
<td>~10.0%</td>
<td>10 mg/m³ (total dust)</td>
<td>Irritating to eyes, respiratory system and skin.</td>
</tr>
<tr>
<td>DMSO</td>
<td>67-68-5</td>
<td>~4.0%</td>
<td>15 mg/m³ (total dust)</td>
<td>Irritating to eyes, respiratory system and skin.</td>
</tr>
</tbody>
</table>

FIRST-AID MEASURES

EYES: Flush with water for 15 minutes. Seek medical advice if irritation persists.
SKIN: Wash with soap and water. Seek medical advice if irritation persists.
INHALATION: Remove the victim from exposure and move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Keep victim quiet and warm. Seek immediate medical attention.
INGESTION: Drink water and seek immediate medical attention. Avoid alcoholic beverages. Never give anything by mouth to an unconscious person.

FIRE-FIGHTING INFORMATION

Use suitable extinguishing agent. Wear NIOSH (or equivalent) approved self-contained breathing apparatus. For small fires only, use carbon dioxide, dry powder or foam. Extinguishing media may produce toxic fumes in burning material. Avoid breathing smoke. Use water spray to cool containers. Spill area should be kept away from ignition sources. Release automatic fire extinguishing systems if necessary. Do not use water.

ACCIDENTAL RELEASE MEASURES

Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NOSH-approved respirator. Cover with dry, non-sparking tools. Use adequate ventilation.

HANDLING AND STORAGE

Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NOSH-approved respirator. Keep container tightly closed. Avoid contact of material with skin or eyes. Use adequate ventilation. Store away from ignition sources and excess heat. Store at -20°C away from incompatible materials. Keep container tightly closed.

PERSONAL PROTECTION

Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NOSH-approved respirator. A qualified industrial hygienist should evaluate the need for respiratory protection.

PHYSICAL AND CHEMICAL PROPERTIES


STABILITY AND REACTIVITY

Product is stable under normal conditions. Incompatible with acid chlorides, phosphorus halides, strong bases, strong oxidizing agents including hydrogen peroxide, peroxides, peroxycarboxylic acids and peroxycarboxylic acid esters, peracids, persulfates and hypochlorites. Keep container tightly closed. Exposure to heat, water and moisture may cause polymerization. Avoid contact of material with skin or eyes. Use appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NOSH-approved respirator. Do not breathe vapour.

TOXICOLOGICAL INFORMATION

EYES: Contact may cause irritation and slight corneal injury. May cause chemical conjunctivitis.
SINUS: Prolonged contact may cause irritation and/or allergic reaction. May cause chemical conjunctivitis.
INHALATION: May cause irritation of gastrointestinal tract with nausea, vomiting and diarrhea.
INGESTION: May cause irritation of gastrointestinal tract with nausea, vomiting and diarrhea.

EFFECTS OF OVEREXPOSURE:

Systemic: Prolonged contact may cause irritation and/or allergic reaction. May cause chemical conjunctivitis.
ADDITIONAL INFORMATION:
Dimethyl Sulfoxide may produce a garlic smell on the breath and body. May cause liver and kidney damage. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties.
Dimethyl Sulfoxide is readily absorbed through the skin and may carry such materials into the body. Only select RTECS information is provided here. Please see actual RTECS entries for complete information.

Irritation, mutation, reproductive effects and toxicity data for Glycerol listed in RTECS under MA8050000. Irritation data: Skin Rabbit = 500 mg/24H Mild (1986). Eye Rabbit = 500 mg/24H Mild (1986). Toxicity data: Oral Rat LD50 = 12600 mg/kg (1945). Inhalation Rat LC50 = >570 mg/m3/1H (1973).
Reproductive effects, irritation, tumorigenic, mutation and toxicity data for Dimethyl Sulfoxide listed in RTECS under PV6210000.
Irritation data: Skin Rabbit 500 mg/24H = Mild (1986). Eye Rabbit 500 mg/24H = Mild (1986).
Toxicity data: Oral Rat LD50 = 14500 mg/kg (1969). Skin Rat LD50 = 40 gm/kg (1967).
Tumorigenic data: Equivocal tumorigenic agent by RTECS criteria (1984).
Reproductive: Effects on embryo or fetus included fetotoxicity (except death, e.g. stunted fetus) (1993) and fetal death (1966). Specific developmental abnormalities - musculoskeletal system (1993), central nervous system (1969), and craniofacial (including nose and tongue) (1966). Fertility - abortion and litter size (e.g. number of fetuses per litter; measured before birth) (1967).
Definition(s): RTECS = Registry of Toxic Effects of Chemical Substances.
ACGIH = American Conference of Governmental Industrial Hygienists.
OSHA = Occupational Safety and Health Administration.

ECOLOGICAL INFORMATION
No information available.

DISPOSAL CONSIDERATIONS
Dispose of material in accordance with applicable local, state, federal regulations.

TRANSPORTATION INFORMATION
US DOT / IATA: No applicable information.

REGULATORY INFORMATION
RCRA - No applicable information. SARA 302 - This material does not have an RQ or TPQ. SARA 313 - This material is not reportable under 313. EPA TSCA Section 8(b) - For Glycerol and Dimethyl Sulfoxide: Chemical Inventory, Exposure Limits - For Glycerol: ACGIH TLV TWA: 10 mg/m3 (total particulate). OSHA PEL TWA: 15 mg/m3 (total dust).
California Proposition 65 - No applicable information.

This data sheet is based upon information believed to be reliable. The Company makes no statement or warranty as to the accuracy or completeness of the information contained herein which is offered for your consideration, investigation and verification. Any use of the information contained in this data sheet must be determined by the user to be in accordance with appropriate applicable regulations.