FT-UPAL322

UptiTherm™ Real Time Probes Kit

Allows the preparation and optimisation of amplification mixtures specifically for their use in Real Time applications.

Research Use Only
Not for use in diagnosis procedures

Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Uptima does not encourage the unlicensed use of patented applications.

Product Description

UptiTherm™ Real Time Probes Kit for labeled probes

Catalog Number	Reactions
UPAL322A	100
UPAL322B	200
UPAL322C	500

STORAGE AND HANDLING INSTRUCTIONS

Upon receipt, store the different reagents under the recommended conditions (-20ºC).

Use non frost-free freezers. Also, for frequent use (more than once a week), aliquot the contents of the vials in different tubes, in order to avoid multiple freeze-thaw cycles.

Do not use the kit after its expiration date. If stored under the recommended conditions, the product will maintain performance through the indicated date on the label. Do not mix reagents from other kits and/or other lots. Discard any residual amount of reagents after using the kit.

Scientific and Technical Information

During last years, real time amplification has become an essential tool for identification and quantification of DNA sequences, specially in those cases in which the sequences are below the detection limit of conventional identification techniques. That is why a great number of methods and instruments have been developed to automate the identification and quantification process.
UptiTherm™ Real Time Probes Kit allows the preparation of reaction mixtures optimised for real time amplification. The kit can be used with a wide variety of instruments, including those amplification systems in capillary tubes with air heating systems, Cepheid tubes or conical tubes used in air or block heating systems.

Materials provided

All the reagents included in UptiTherm™ Real Time Probes Kit are presented in solution. The reaction mixture is prepared just before use and dispensed to the different amplification tubes.

- **Mix Buffer A**
  Store at –20ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly.
- **$MgCl_2$ (50 mM)**
  Store at –20ºC. Thaw and handle on ice. Mix thoroughly before use. Do not freeze/thaw repeatedly.
- **UptiTherm™ DNA polymerase (1 U/µl)**
  Store at –20ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly.
- **dNTP Mix (10 mM)**
  Store at –20ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly.
- **Mix Buffer B**
  Store at –20ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly

Directions for Use

Pre-Protocol Considerations :

NOTE: Please read carefully the 'Warnings and Precautions' chapter before proceeding. Handle all reagents and samples carefully in order to avoid false positive results due to contamination.

NOTE: The DNA condition is a key point to obtain optimal results. Samples should be transported and stored frozen (at -20 ºC or at -80ºC). In samples that have been stored without refrigeration, DNA can be degraded and its recommended to perform the analysis shortly after the extraction. The reproducibility of the quantification results of a sample at different storage times will depend on the storage conditions and will be more affected when storing without refrigeration. In case of working with clinical samples, handle all them as if they are capable of transmitting infectious agents.Transportation of clinical samples must comply with country, federal, state and local regulations for the transport of etiologic agents.

NOTE: A kit for DNA purification is included in the kit only upon demand. Methods for DNA purification can be either phenol-based or resin-based, provided they yield enough amount of pure DNA.

Protocol

DNA amplification is performed with a thermostable DNA Polymerase from *Thermus*. In the presence of magnesium, and with the suitable salt and ionic strength conditions, the enzyme shows DNA polymerisation activity, using as anchor sequence-specific primers and a DNA molecule as template.

DNA is purified from the sample to be analysed, and added to the reaction mix in the corresponding amplification vial, where the amplification process will take place.

NOTE

This product is not recommended for amplifications involving sequences homologous to E. coli

NOTE

Mix the reaction components in the laminar flow cabinet. In order to avoid contaminations, never introduce DNA in the laminar flow cabinet.

The amplification must be started in the next 10 minutes after having added the purified DNA and controls to the amplification mix.

For optimal results, it is essential to keep the reaction vials refrigerated until their introduction in the thermal cycler. When working with standard conical amplification vials, be sure to keep them on ice or in coolers, avoiding wetting the optical cap. If capillary vials are employed, make sure that the cooler has been at 4ºC at least for 4 hours before use. Use of reaction mixes and vials in non-refrigerated conditions may cause a drastic decrease in the sensitivity and quality of the obtained fluorescence curves.

Do not touch nor wet the vial surfaces through which the fluorimetric detection is performed.

Check thermal cycler regularly. Non existent calibration or inadequate maintenance of the equipment may result in erroneous results.

PROTECT PROBE VIALS FROM LIGHT AT ALL TIMES

1.A- Amplification mixtures preparation

1.- Determine the number of samples to be analysed, including the problem samples and the standard curve, if quantification is performed. It is highly recommend to include at least one positive and one negative control in each experiment.
2.- Place the tubes in the laminar flow cabinet and mix the reaction mix gently by inversion
3.-When required (e.g. ABI PRISM thermal cyclers), prepare fresh dilutions of the reference dye (ROX) prior to setting up the reactions. Keep all tubes containing the reference dye protected from light at all times.

1.B. Amplification mixtures:

Amplification conditions may vary depending on the amplification system and the instrument used for the assay.

Keep all the reagents on ice until their introduction in the thermal cycler.

4.- Prepare a master mix taking into account the following proportions

REAGENTS INCLUDED IN THE KIT

Mix Buffer A	2.0 µl
$MgCl_2$ (50 mM)	1.6 µl
dNTPs (10 mM)	0.4 µl
DNA Polymerase (1U/µl)	1.0 µl

NOTE

For some experiments, use of 2 U (i.e. 2µl) is recommended. If the experiment renders no results with 1 µl, use 2 µl or higher amounts of polymerase.

5.- Add DNA, Mix Buffer B and primer up to a final volume of **20 µl**. In case of larger reaction volumes, scale the indicated amounts, keeping the proportion between components (specially important with Mix Buffer A)

REAGENTS NOT INCLUDED IN THE KIT (recommended amount)

Amplification primers	3 µM – 1 µM (final concentration)
Taqman ® /Scorpion® probes	(follow the manufacturer's instructions)
DNA template	(depends on the experimental assay)
Reference dye (ROX)[1]	(follow the manufacturer's instructions)

6.- Gently mix the reaction mixture without creating bubbles. Do not vortex.
7.- Centrifuge the reactions briefly if needed (e.g. SmartCycler)
8.- Close the amplification vials and place them in the thermal cyclers.

NOTE

This protocol has been optimised for the following Real time quantification equipments: LightCycler (Roche), i-Cycler (Biorad), SmartCycler I and II (Cepheid), Rotor-Gene 3000 (Corbett Research) and ABI PRISM 7000 series (Applied Biosystems). For other thermal cyclers, optimisation of the reaction parameters may be required. Please contact our Technical Department (uptima@interchim.com).

1.C. Reaction mixtures optimisation parameters.

When an optimisation of the amplification reaction is required, we suggest to consider the following recommendations:

- Assay a standard curve of $MgCl_2$ concentrations: Recommended range: 4-6 mM
  (a final concentration of 4mM is obtained when using the kit).
- Modify the annealing/extension temperatures and times, as well as the slopes between the amplification cycles.
- Modify the concentration of the primers.
- In some cases it may be necessary to modify the volume of Taqman ® probes

1.D. Additional notes.

- Experimental samples may have different DNA concentrations. The quantity of template DNA to be added in each reaction depends on its purity and the experimental system used in every case, Therefore we recommend to determine empirically this quantitative by e.g. $A_{260/280}$ measurements.

In case that the extracted DNA can not be quantified we recommend to add a fixed volume of the extraction mixture to the problem samples. The purpose of this recommendation is to obtain comparable quantitative results.

2.- Interpretation of results

NOTE

[1] Some manufacturers recommend its use (e.g. ABI PRISM-Applied Biosystems) for the normalisation of the fluorescence signal during Real time DNA amplification.

The use of thermal cyclers with coupled fluorescence capture systems, allows the Real time product monitoring at each amplification cycle. The interpretation of the results is performed with the help of specific software. Follow therefore the instructions and advice provided by the manufacturer.

3.- Quality Control

It is recommended to run at least one Positive and one Negative Control each time the test is performed. As with any new laboratory procedure, new users should consider performing additional controls (both positive and negative), until a high degree of confidence is reached.

MATERIALS REQUIRED BUT NOT PROVIDED

Pre-amplification area

- Automatic pipettes[2] (10, 20 and 200 µl), positive-displacement or aerosol resistant pipette tips, RNase-free [3]
- Disposable examination gloves, powder-free
- Screw cap polypropylene tubes, 1.5 ml capacity, non siliconised, conical, sterile, RNase-free. It is recommended to use screw cap tubes, in order to avoid the potential contamination of samples and controls.
- Racks for 1.5 ml vials

Amplification area

- Real-time thermal cycler (LightCycler (Roche), i-Cycler (Biorad), SmartCycler (Cepheid), Rotor-Gene 3000 (Corbett Research) or ABI PRISM (Applied Biosystems)). The use of this kit in other equipments has not been tested. For further information, contact our Technical Dpt. (uptima@interchim.com).
- Laminar flow cabinet
- Racks for reaction vials
- Real time amplification vials (depends on the equipment).
- Positive-displacement or aerosol resistant pipette tips, RNase-free
- Disposable examination gloves, powder-free
- Termi-DNA-Tor (Cat. No. S54860) or equivalent

WARNINGS AND PRECAUTIONS

DNA amplification allows the amplification of minute quantities of template from a sample in an exponential manner. However, this means that foreign DNA present in the environment may also be amplified. Therefore, special laboratory practices are necessary in order to avoid false positive amplifications.
The list below contains several warnings and precautions that must be considered. For detailed information, we recommend to read the Material Safety Data Sheet (MSDS). Please contact our Technical Department for additional information. (uptima@interchim.com).

A. Use of dedicated micropipettes in each area (sample preparation, amplification and pre-amplification) is highly recommended.

B. We recommend to use filter tips in order to avoid cross contamination. Pipettes must be regularly checked, in order to ensure that they are accurate within 3 % of stated volume. Use different micropipettes depending on the aliquoted volume.

[2] Precision of automatic pipettes must be in the range of 3 % of the indicated volume. If necessary, callibrate and check regularly, following manufacturer's instructions. It is recommended to use RNase-free filter tips and positive displacement tips, in order to avoid cross contamination between samples and amplicons.
[3] It is recommended to use different sets of pipettes for each reaction step (pre-amplification, amplification), in order to avoid contaminations that may render false positive results.

C. Negative results may occur due to the Polymerase inhibition. DNA purification must proceed in such a way that enough amount of pure DNA is obtained. It is recommended to check suitability of DNA preparations for the amplification (i.e. performance of an amplification for detection of human DNA).

D. Follow general instructions for laboratory safety (e.g. do not eat, drink or smoke in laboratory work areas, wear disposable gloves, wear clean lab coats and eye protection, wash hands thoroughly after handling specimens and test reagents, etc.).

E. Open and close reagent vials carefully. Follow temperature and light exposure instructions. After use, close vials and store at indicated temperatures.

F. Do not use a kit after its expiration date.

G. Extreme care must be taken when aliquoting the different volumes in each reaction step. Mix well after addition of each reagent, unless otherwise noted. Read instructions for use of automatic pipettes .

H. Do not pipette by mouth.

I. Packaging material included within the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of the kit reagents.

J. Plastic material included within the kit is resistant under normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, the impossibility to use the kit.

K. Laboratory workflow must be unidirectional, from pre-amplification to amplification area. Specific equipment for each working area must be used, in order to avoid cross contaminations. Equipment used for amplification must remain in this area at all times.

L. Gloves must be worn in each area and must be changed before leaving that area.

M. As with any test procedure, good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents. Discard any reagents that may be suspect for their purity.

N. Do not touch or wet the vials in the detection areas. Use non talcum powder gloves.

WARRANTY

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Uptima' obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Uptima' expense, of any products which shall be defective in manufacture, and which shall be returned to Uptima, transportation prepaid, or at Uptima option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

The product has been designed for research use only, and to be used by qualified professionals only. It is the user's responsibility to ensure that a given product is fit for a given application. Any product that does not meet the performance standards stated in the product specification sheet will be replaced at no charge. This warranty limits our liability to the replacement of the product. No other warranties of any kind, express or implied, including, without limitation, implied warranties for merchantibility or fitness for a particular purpose, are provided by Uptima. Uptima shall have no liability for any direct, indirect, consequential or incidental damages arising out of the use, the results of use or the inability to use any product.

Trademarks:

ABI PRISM ® is a registered trademark of PE Corporation
Applied Biosystems ® is a registered trademark of Applera Corporation
GeneAmp ™ is a trademark of Applied Biosystems Inc.
ICycler ™ is a trademark of Bio-rad Laboratories, Inc
Rotor-Gene 3000™ is a trademark of Corbett Research Limited
ROX ™ s a trademark of Applera Corporation
SmartCycler® is a registered trademark of Cepheid
Termi-DNA-Tor™ is a trademark of Biotools, Biotechnological & Medical Laboratories, S.A.
TaqMan® is registered trademark of Roche Molecular Systems, Inc
Scorion® is registered trademark of Dxs Ltd.

Other Information

Related Products

Product	Catalog Number	Quantity
UptiTherm DNA Polymerase, 5 U/µl with Mg free Buffer + 50 mM MgCl2 buffer	UPS53921	1000 Units
UptiTherm DNA Polymerase, 5 U/µl with 2 mM MgCl2 Buffer	UPS53881	1000 Units
UptiTherm Master Mix in PCR tube, Mg buffer	UPS54071	50 x 0,2 ml
UptiTherm Master Mix in PCR tube, Mg free	UPS54081	50 x 0,2 ml
UptiPfu DNA Polymerase, 1U/µl with Mg free Buffer + 50 mM MgCl2 buffer	UPAK5105	500 Units
UptiPfu DNA Polymerase, 1U/µl with 2 mM MgCl2 Buffer	UPAK5102	500 Units
UptiTherm Hot Start PCR Master Mix	UPQ6587A	250 Units
RedTaq DNA Polymerase, Mg free	UPAP1221	1000 Units
Rapid Ligation Kit	UPN14171	50 Reactions
RT-PCR One Tube	UPS53944	100 Reactions
PCR Optimer Kit	UPAP2400	3 x 1.5 ml
Enhancer PCR Optimer Kit	UPAP2410	10 reactions
dNTP Set (dATP, dGTP, dCTP, dTTP, 100 mM each)	UP968640	4 x 250 µl
PCR Mix 3 (10 mM of each dATP, dGTP, dCTP, dTTP)	UP984440	200µl
GC5 Value Efficiency, 10^8Cfu/µg pUC19 Chemically Competent Cells	UPAM893A	10x 200µl
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells	UPAM889B	10x 50µl
SuperPath GC10, 10^{10} Cfu/µg pUC19 Electro Competent Cells	UPAM885A	5x 80µl
SOC Medium	UPAN146A	10x 10ml