

PRODUCT NAME
<b>Universal Mastermix 7,5ml</b>
Please refer to the Certificate of Analysis for the expiration date and the lot number <b>FOR IN VITRO USE ONLY</b>
<b>Cat. No. DMMLD2D600</b>

#### Product description

The Universal Mastermix is a Hotstart Mastermix designed to simplify the preparation of a real-time PCR assay. The Universal Mastermix contains a Hotstart Taq NA polymerase, optimised reaction buffer, 4mM (final concentration) MgCl<sub>2</sub> and nucleotides (dNTPs). In separate tubes, passive reference (ROX), SYBR® Green, and FITC.

#### Storage and stability

Store the Universal Mastermix at -15 to -25°C in a constant temperature freezer. When stored under these conditions the reagents are stable for a minimum of 12 months.

#### Kit Contents

The Universal Mastermix contains enough PCR reagents for up to 600 reactions (25µl)

Reagent	Volume
2X Reaction Buffer dNTPs (including dUTP), Hotstart DNA polymerase, MgCl <sub>2</sub> (4mM final concentration)	7.5ml
Passive reference Pink Cap, 500µl stock solution	0.5ml
SYBR® Green Brown tube	0.5ml
1mM FITC solution Green Cap, 1000µl stock solution	1 ml

Depending the applications and the system prepare a **stock solution** following the instructions in the table enclosed. (Universal Mastermix). This stock solution will be used to prepare the PCR Mastermix (see next page).  
Passive reference stock solution for systems which require passive reference.  
FITC for Biorad users working with SYBR® Green.

#### Materials Required But Not Supplied

The following items will be required in addition to the reagents supplied in the Universal Mastermix.

#### Reagents :

Deionised or distilled water  
Unlabelled primers

#### Materials :

Optical 96-Well Reaction Plate  
Optical Tubes and Caps as needed  
Microcentrifuge  
Polypropylene tubes  
Pipettes, preferably positive displacement  
Pipette tips, with filter plugs  
Vortex  
Disposable Gloves

**FOR SYBR GREEN**

**Prepare a stock solution by adding**

System	Master Mix 2X	Rox	SYBR Green	FITC	Denaturation time	PCR cycles	Normal Cycles	Fast Cycles
7000-7300-7900 (ABI)	1000µl	40 µl	60 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	5' at 95C; 60' at 60C (7900 ONLY)
7500 (ABI)	1000µl	2.8 µl	60 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	5' at 95C; 60' at 60C
LC480 (Roche)	1000µl	0 µl	60 µl	0 µl	10 minutes at 95C	40		5' at 95C; 60' at 60C
Icycler (Biorad)	1000µl	0 µl	60 µl	10µl of a 1000 dilution of the FITC solution	10 minutes at 95C	40	15' at 95C; 60' at 60C	
Rotorgene 6000 (Corbett)	1000µl	0 µl	60 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	

Use 12.5 µl of this stock solution and add primers and DNA to reach 25µl

**FOR DOUBLE DYE PROBE TAQMAN™**

**Prepare a stock solution by adding**

System	Master Mix 2X	Rox	SYBR Green	FITC	Denaturation time	PCR cycles	Normal Cycles	Fast Cycles
7000-7300-7900 (ABI)	1000µl	40 µl	0 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	5' at 95C; 60' at 60C (7900 ONLY)
7500 (ABI)	1000µl	2.8 µl	0 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	5' at 95C; 60' at 60C
LC480 (Roche)	1000µl	0 µl	0 µl	0 µl	10 minutes at 95C	40		5' at 95C; 60' at 60C
Icycler (Biorad)	1000µl	0 µl	0 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	
Rotorgene 6000 (Corbett)	1000µl	0 µl	0 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	
Stratagene Mx3000P/ 3005P	1000µl	0 µl	0 µl	0 µl	10 minutes at 95C	40	15' at 95C; 60' at 60C	

Use 12.5 µl of this stock solution and add primers and DNA to reach 25µl

### PCR Reaction Mix

A typical 25 µl PCR Reaction Mix would contain the following:

Component	Volume (µl)	Final concentration (in 25µl)
<b>2x Stock solution</b>	12.5	1x
Forward primer	2.5	0.1µM
Reverse primer	2.5	0.1µM
Probe when required	2.5	0.3µM
Water	x	-
Template	5	-
<b>Total Mix</b>	<b>25µl</b>	

### PCR Temperature Cycling Conditions

ATTENTION 10min initial deprotection time:

Normal cycles	
Hotstart enzyme Activation	10min. 95°C
45 Cycles	15sec. 95°C 60sec. 60°C
Hold	25°C forever
Fast cycles	
Hotstart enzyme Activation	10min. 95°C
40 Cycles	5sec. 95°C 60sec. 60°C
Hold	25°C forever

### Quality Control

The Universal Mastermix has been tested for performance on an authorised real-time sequence detector. Products and procedures described in this protocol are intended for research purposes only.

### Notice to purchaser : limited license

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#### Diagenode sa. BELGIUM | EUROPE

LIEGE SCIENCE PARK  
Rue Bois Saint-Jean, 3  
4102 Seraing (Ougrée) - Belgium  
Tel: +32 4 364 20 50  
Fax: +32 4 364 20 51  
orders@diagenode.com  
info@diagenode.com

#### Diagenode Inc. USA | NORTH AMERICA

400 Morris Avenue, Suite 101  
Denville, NJ 07834 - USA  
Tel: +1 862 209-4680  
Fax: +1 862 209-4681  
orders.na@diagenode.com  
info.na@diagenode.com

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