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Thermo Scientific ABsolute QPCR SYBR Green Capillary Mix

Description

ABsolute™ QPCR SYBR® Green Capillary Mix has been developed to quantify DNA and cDNA*. With the exception of primers and template, this 2X mix contains all the components required to perform a rapid, sensitive and reproducible QPCR reaction:

- Thermo-Start™ DNA Polymerase, a chemically modified hot-start version of Thermoprime Plus DNA Polymerase, which prevents non-specific amplification during the reaction set-up. **This enzyme requires an activation step at 95°C for 15 minutes.**
- Proprietary reaction buffer which provides highly sensitive, specific and consistent fluorescence readings for real-time and end-point analysis. This buffer has been optimized for $MgCl_2$ and enhancers to improve amplification across a wide range of templates including plant DNA and GC rich fragments.
- dNTP's, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.
- SYBR® Green I, a dye which fluoresces after binding of the double-stranded DNA. The overall fluorescence increases proportionally to the double-stranded DNA concentration.

Kit Content

Vial contents	Pack Size (cap color)		
	A	B	C
ABsolute QPCR SYBR Green Capillary Mix (2X)	2 x 1.25ml (green)	16 x 1.25ml (green)	5ml (clear)
$MgCl_2$ (1 M)	100µl (clear)	2 x 100µl (clear)	100µl (clear)

Cycler Compatibility

ABsolute™ QPCR SYBR® Green Capillary Mix is compatible for use with any capillary format cyclers, including the Roche Lightcycler® 2.0.

* **For RNA template**, use Verso™ SYBR® Green 1-Step QRT-PCR Capillary Kit (AB-4108)

INFORMATION

Thermo-Start™ DNA Polymerase

The enzyme requires an activation step at 95°C for 15 minutes.

Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

$MgCl_2$

The initial concentration of $MgCl_2$ in the ABsolute QPCR SYBR Green Capillary Mix corresponds to 3 mM in the final 1X reaction. This concentration is effective over a broad range of templates. Some assays may be improved further with $MgCl_2$ optimization. A separate vial of 1 M $MgCl_2$ is therefore supplied with each kit.

$MgCl_2$ concentration can be increased as follows: each 2.5 µl or 10 µl addition of $MgCl_2$ to the 1.25 ml or 5 ml undiluted ABsolute QPCR SYBR Green Capillary Mix respectively corresponds to an increase of 1 mM in the final 1X reaction. Scale up or down accordingly. Mix thoroughly by inverting the vial ten to twenty times. **Do not vortex.**

Storage Conditions

Store at -20°C until ready for use. ABsolute™ QPCR SYBR® Green Capillary Mix is stable for a minimum of 12 months. The reagents can be stored at 4°C for up to 1 month. Avoid repeated freeze thawing. The SYBR® Green dye is light sensitive; exposure should be minimized. Shipped on ice within the UK and on dry ice for international and within the US.

Additional Info

- The use of disposable gloves, DNase and RNase free filter tips and plastics is recommended.
- For optimal results, the recommended amplicon length is in the range of 60 to 300 bp.
- As best performance is achieved with dTTP, the ABsolute QPCR SYBR Green Capillary Mix contains a nucleotide mix with dTTP instead of dUTP.

DIRECTIONS FOR USE

Tips and Protocol

Thaw the reagents on ice. Mix and spin down the solutions before use to recover the maximum amount. **Do not vortex the ABsolute QPCR SYBR Green Capillary Mix.** Briefly centrifuge to avoid bubbles within the wells, as these will interfere with the fluorescence. Always include a no template control (NTC).

Example of Reaction Mix preparation for a 20 µl final reaction:

		Volume	Final Concentration
Reaction Mix	ABsolute QPCR SYBR Green Capillary Mix (2X)	10 µl	1X
	Forward primer (1 µM) [a]	1.4 µl	70 nM
	Reverse primer (1 µM) [a]	1.4 µl	70 nM
	Water (PCR grade) [b]	variable	
	Template (DNA or cDNA) [c]	1 - 5 µl	<250 ng/reaction
	Total volume	20 µl	

Example of a **QPCR thermal cycling program**:

	Temp.	Time	Number of cycle
Enzyme activation	95°C	**15 min**	1 cycle
Denaturation	95°C	15 sec	40 cycles
Annealing [d]	50-60°C	30 sec	
Extension [e]	72°C	30 sec	

It is recommended to perform a melt curve to confirm the specificity of the reaction.
Example of a **melt curve program** [f]:

Denaturation	95°C	30 sec	1 cycle
Starting temp.	60°C	30 sec	1 cycle
Melting step [g]	60°C	10 sec	80 cycles

Notes

a – For optimization, a primer titration should be performed from 50 nM to 300 nM final concentration. Scale up or down the volume and concentration as appropriate.
b – The volume of the total reaction should be completed up to 20 µl with water.
c – The volume of template to add to the QPCR reaction can be adjusted as required. For standard templates only 1 µl should be added to reduce the carryover of any PCR inhibitor. This volume can be increased up to 5 µl for low copy number templates.
d – Annealing temperature dependent on primer sequence.
e – Time of extension depends on the length of the amplicon. If the amplicon exceeds 300 bp amplification time should be adapted (Thermo-Start™ DNA Polymerase extends approximately at 1000 bp/min).
f – Melt curve program may vary depending on instrument manufacturer and software.
g – Increase set point temperature by 0.5°C per cycle.

Quality control

ABsolute QPCR SYBR Green Capillary Mix is tested functionally using QPCR. The product must demonstrate linearity of amplification over a specified serial dilution of human genomic DNA.

Ordering Information

AB-1285/A	ABsolute™ QPCR SYBR® Green Capillary Mix	250 x 20 µl rxns
AB-1285/B	ABsolute™ QPCR SYBR® Green Capillary Mix	2,000 x 20 µl rxns

All formats are supplied with an additional vial of 1 M $MgCl_2$.

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

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