Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific ABsolute qPCR SYBR Green Fluorescein Mix

#AB-1220/A 5 mL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-1219/B 1600 rxns of 25 µL	#AB-1220/A 400 rxns of 25 µL
2X ABsolute SYBR Green Fluorescein Mix	16 × 1.25 mL	5 mL
1 M $MgCl_2$	100 µL	100 µL

Store at -20°C

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Description

Thermo Scientific ABsolute qPCR SYBR® Green Fluorescein 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 Scientific Thermo-Start DNA Polymerase, a chemically modified hot-start version of Thermo Scientific ThermoPrime *Taq* DNA Polymerase, which prevents non-specific amplification during the reaction set-up. Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). **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 to the double-stranded DNA. The overall fluorescence increases proportionally to the double-stranded DNA concentration.
- Fluorescein, passive reference dye for normalization of data.

Cycler Compatibility

ABsolute™ qPCR SYBR Green Fluorescein Mix is compatible with all qPCR cyclers that require fluorescein including the Bio-Rad iCycler® and MyiQ™.

Fluorescein Dye

Fluorescein acts as a passive reference dye to facilitate normalization of data. The concentration of fluorescein in the ABsolute qPCR SYBR Green Fluorescein Mix corresponds to 10 nM in the final 1X reaction.

$MgCl_2$

The initial concentration of $MgCl_2$ in the ABsolute qPCR SYBR Green Fluorescein 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 Fluorescein 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 Fluorescein 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 fluorescein and SYBR Green dyes are light sensitive; exposure should be minimized.

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 Fluorescein Mix contains a nucleotide mix with dTTP instead of dUTP.

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 Fluorescein 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 25 µL final reaction:

	Volume	Final Concentration
2X ABsolute qPCR SYBR Green Fluorescein Mix	12.5 µL	1X
Forward primer (1 µM)*	1.75 µL	70 nM
Reverse primer (1 µM)*	1.75 µL	70 nM
Template (DNA or cDNA)**	1-5 µL	<250 ng/rxn
Water, nuclease-free (#R0581)	To 25 µL	
Total volume	25 µL	

*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.

**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 carryover of PCR inhibitors. This volume can be increased up to 5 µL for low copy number templates.

Example of qPCR thermal cycling program:

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

*Annealing temperature depends on primer sequence.
**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).

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

Denaturation	95 °C	30 s	1 cycle
Starting temp.	60 °C	30 s	1 cycle
Melting step**	60 °C	10 s	80 cycles

*Melt curve program may vary depending on instrument manufacturer and software.
**Increase set point temperature by 0.5°C per cycle.

CERTIFICATE OF ANALYSIS

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

Quality authorized by:  Jurgita Zilinskiene

NOTICE TO PURCHASER:

- Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 6,127,155, 5,677,152 (claims 1 to 23 only) and 5,773,258 (claims 1 and 6 only), 5,994,056, 6,171,785. Use of this product in a passive reference method is covered by the following U.S. Patent: 5,928,907 (claim numbers 12-24, 27-28) and corresponding patent claims outside the US. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.
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Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.