

## Titan HotTaq EvaGreen® HRM Mix (no ROX)

Cat. No.	Pack Size
BTS0022	100 µl (25 reactions) SAMPLE
BT11202	1 ml (250 reactions)

### Application:

High Resolution Melt (HRM)

### Reagent Composition:

- Titan HotTaq DNA polymerase
- 5 x EvaGreen® HRM buffer
- dNTPs
- 12.5 mM MgCl<sub>2</sub>  
1 x PCR solution – 2.5 mM MgCl<sub>2</sub>
- EvaGreen® dye
- BSA
- No ROX dye

### Description:

Titan HotTaq EvaGreen® HRM Mix (no ROX) is an optimised ready-to-use solution for High Resolution Melt (HRM) Analysis, incorporating EvaGreen® dye. It comprises all the components necessary to perform qPCR and HRM Analysis: Titan HotTaq DNA Polymerase, ultrapure dNTPs, MgCl<sub>2</sub> and EvaGreen® dye. Only water, template and primers need to be added.

Titan HotTaq DNA Polymerase is activated by a 12 min incubation step at 95°C. This prevents extension of nonspecifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

### EvaGreen® Dye:

EvaGreen® is a DNA-binding dye with many features that make it superior for HRM. Apart from having similar spectra, EvaGreen® has three important features that set it apart from SYBR® Green I: EvaGreen® has much less PCR inhibition, is an extremely stable dye and has been shown to be non-mutagenic and non-cytotoxic. EvaGreen® is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM!

### Shipping and Storage conditions:

Routine storage: -20°C

Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of Titan HotTaq EvaGreen® HRM Mix (no ROX).

### Recommendation:

Reaction setup at room temperature is highly recommended for Titan HotTaq EvaGreen® HRM Mix.

### Recommended qPCR reaction mix:

Component	Volume	Final conc.
5 x Titan HotTaq EvaGreen® HRM Mix (no ROX)	4 µl	1x
Primer Forward (10 pmol/µl)	0.16-0.5 µl	80-250 nM
Primer Reverse (10 pmol/µl)	0.16-0.5 µl	80-250 nM
DNA template <sup>1</sup>	variable <sup>1</sup>	variable <sup>1</sup>
H <sub>2</sub> O PCR grade	up to 20 µl	
<b>Total</b>	<b>20 µl</b>	

<sup>1</sup> Conc. of cDNA 0.1 pg/μl - 10 ng/μl; gDNA 10 pg/μl – 4 ng/μl

#### Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
Initial activation <sup>2</sup>	95°C	12 min	1
Denaturation	95°C	15 s	40
Annealing	60°-65°C	20 s*	
Elongation	72°C	20 s*	

<sup>2</sup> **IMPORTANT:** To activate the polymerase, include an incubation step **at 95°C for 12 minutes** at the beginning of the qPCR cycle.

For HRM step please use settings recommended by producer.

#### Safety warnings and precautions:

For *in vitro* use only

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing such as laboratory overalls, gloves and safety goggles. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, immediately rinse with plenty of water.

*Some applications this product is used in may require a license which is not provided by the purchase of this product. Users should obtain the license if required.*

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