

5x Hot Start EvaGreen[®] HRM Mix (no ROX)

Cat. No.	Pack Size	Conc. (MgCl ₂)
29417	0.2 ml SAMPLE (50 reactions)	12.5 mM
29418	1 ml (250 reactions)	12.5 mM

For *in vitro* use only

Lot nr:

Exp. Date:

Description:

5x Hot Start 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: Hot Start Taq DNA Polymerase, ultrapure dNTPs, MgCl₂ and EvaGreen[®] dye. The user simply needs to add water, template and primers.

Hot Start Taq DNA Polymerase is activated by a 15 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

Applications:

- High Resolution Melt (HRM)

Mix Composition:

- Hot Start Taq DNA Polymerase
- 5x EvaGreen[®] HRM buffer
- 12.5 mM MgCl₂
1x PCR solution – 2.5 mM MgCl₂
- dNTPs, including dTTP to improve reaction sensitivity and efficiency compared to dUTP
- EvaGreen[®] dye
- BSA
- No ROX dye

EvaGreen[®] Dye:

EvaGreen[®] is a DNA-binding dye with many features that make it a superior alternative to SYBR[®] Green I for qPCR. 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 extremely stable dye and has been shown to be nonmutagenic and noncytotoxic. 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 5x Hot Start EvaGreen[®] HRM Mix (no ROX).

Recommended qPCR reaction mix:

Component	Volume	Final conc.
5x Hot Start EvaGreen [®] HRM Mix	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	1-5 µl	0.01-10 ng/µl
H ₂ O PCR grade	up to 20 µl	
Total	20 µl	

Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
Initial denaturation	95°C	15 min	1
Denaturation	95°C	15 s	40
Annealing	60°-65°C	20 s	
Elongation	72°C	20 s	

IMPORTANT: To activate the polymerase, follow the incubation step **at 95°C for 15 minutes** at the beginning of the qPCR cycle.

For HRM Step please follow the guidelines from your qPCR/HRM platform manufacturer.

Safety warnings and precautions:

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 glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

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