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Eva488 Lyophilized qPCR Kit manual

Contents

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Eva488 Lyophilized qPCR Kit manual

Eva488 lyophilized qPCR kit is designed to determine the precise content of the DNA matrix in the test sample. It is applicable for gene copy number detection, gene expression analysis, and genotyping using the Real-Time qPCR (RT-qPCR) method. The kit contains hot start (HS) polymerase, which prevents nonspecific amplification. It uses intercalating Eva488 dye (EvaGreen® analog), that bounds to double-stranded DNA without inhibiting the reaction and exhibits a high fluorescence signal increase.

Eva488 lyophilized qPCR kit reaction mix does not contain ROX reference dye; thus, it is compatible with any real-time DNA amplifier. A reaction mixture volume of 1 mL is good for 100 rxn of 20 µL.

Kit components

Kit component	Count
	33162 **100 reactions**
31315, Lyophilized Taq polymerase composition with hot start, 100 rxn	1
52215, Polymerase reconstitution buffer with Eva488, 1 mL	1

Transportation: at room temperature for up to 3 weeks. Store at -20 °C.

Shelf life 12 months.

Compatibility with equipment: compatible with any thermocyclers.

Protocol

1. Dissolve **HS Taq polymerase** (Component #1) in **Polymerase reconstitution buffer containing Eva488 dye** (Component #2). For this, add the whole volume of the Component #2 vial into the Component #1 vial. Resuspend carefully and wait until completely dissolved (~5 minutes).

   Important! *After preparing, store the reaction mixture at -20 °C.*

2. Prepare a reaction mixture according to the table in the proportion per (N+0.1N) reactions, where N is the required number of reactions. Add components in the given order. Carefully vortex the resulting blend, and spin down the solution using centrifuge.

   Important! *The volume of the reaction solution may vary depending on the specific application, but a volume of less than 10 µL is not recommended.*

   Calculations for the total volume 20 µL per single reaction:
   The reaction components should be recalculated with the proportion below when using a different reaction volume.

Component	Volume	Note
2x Reaction mix	10 µL	—
Forward primer	0.5–1.5 µL 10 µM solution	5–15 pmol/reaction (final concentration 250–750 nM)
Reverse primer	0.5–1.5 µL 10 µM solution	
Deionized water	Is added to bring to the reaction volume of 20 µL	
DNA	2–9 µL (cDNA, 50–100 ng genomic DNA, 1–100 pg plasmid DNA)	Is added separately in each vial (see step 3)
The total volume of reaction	**20 µL**	

3. Add the final volume of the reaction mixture in separate PCR tubes without taking into account the volume of the DNA sample. Add DNA samples to each PCR tube, and spin down the solution using centrifuge.

Important! *To obtain reliable and reproducible data, run the PCR reaction at least two times for each DNA sample.*

Amplification program

Use the standard method to calculate the primers' melting point (Tm) using Nearest-Neighbor algorithm (SantaLucia J. Jr., 1998). The primer annealing temperature is determined according to the equation: Ta=Tm–5 °C.

If the annealing temperature of the primers ≥60 °C:

Stage	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 minutes	1
Denaturation	95 °C	10 seconds	40
Primers annealing combined with elongation (Fluorescence detection should be performed at this stage)	60–72 °C	30–60 seconds	

If the annealing temperature of the primers <60 °C:

Stage	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 minutes	1
Denaturation	95 °C	10 seconds	40
Primers annealing (Fluorescence detection should be performed at this stage)	55–59 °C	10–15 seconds	
Elongation	72 °C	15–30 seconds	

Important! *After amplification, we recommend performing amplicon melting in a range from 60 up to 95 °C to ensure the absence of nonspecific amplification.*

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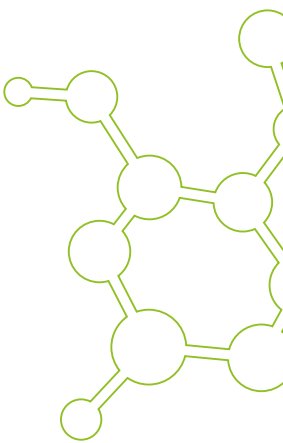

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