GENAXXON bioscience

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Red MasterMix Fluoro (2X) with gel staining dye

3-in-1 formula of PCR mastermix, loading dye and nucleic acid staining dye to simplify the PCR workflow to a single preparation step

Component	Cat#	M3236.0100	M3236.0500	M3236.2500	Colour code of cap
Red MasterMix Fluoro (2X) with gel staining dye		2x 1.25mL	10x 1.25mL	50x 1.25mL	

Product description

Genaxxon's Red MasterMix Fluoro (2X) is a ready-to-use 3-in-1 PCR reaction mixture - simply add primers and templates. The Red MasterMix Fluoro (2X) is a pre-mixed solution containing a PCR master mix (Taq DNA polymerase, PCR buffer, dNTPs in optimal concentrations) with a red loading dye for visual control of the pipetting steps and an additional fluorescent nucleic acid staining dye for fast and easy detection of the DNA bands. The Taq DNA polymerase has a 5'-3' exonuclease activity but lacks the 3'-5' exonuclease activity.
The Red MasterMix Fluoro (2X) contains an additive red loading dye which allows subsequent electrophoresis without adding a loading buffer. After electrophoresis, the included fluorescent nucleic acid staining dye allows an instant band visualisation using a blue-light transilluminator or a UV illuminator without adding an additional gel staining dye. This makes our Red MasterMix Fluoro (2x) even more time- and cost-saving than our proven Red MasterMix (2x) Fluoro. The included fluorescent dye is a safe, non-toxic and non-mutagenic alternative to ethidium bromide.
The Red MasterMix Fluoro (2x) with fluorescent dye was developed for use in routine PCR up to 4 kb amplicon length. The special composition of the buffer guarantees reproducible results even after repeated thawing and freezing cycles. Our Red MasterMix (2x) Fluoro is shipped in convenient aliquots of 1.25mL.

Features

- Simplify your PCR workflow to a single preparation step
- No post-staining processing of nucleic acid
- Direct loading onto agarose gel for electrophoresis without adding a loading buffer
- High degree of sensitivity (comparable to ethidium bromide)
- No destaining requirement
- Use the blue light or UV to detect the signal

Product Specifications

2-time ready-to-use master mix for PCR including a red dye for better visualization of pipetting and as a gel loading dye as well as a fluorescence nucleic acid staining dye for detection of DNA bands in an electrophoresis gel. dNTPs, buffer and DNA polymerase already included.
Buffer contains orange G & xylene cyanol FF as tracking dyes.
Buffer contains a fluorescent gel staining dye for visualization of DNA bands.

Source

Recombinant, purified from *E.coli*

Product quantity

Genaxxon's Red MasterMix Fluoro (2X) with gel staining dye is supplied as a convenient 2x master mix, including a red tracking dye for electrophoresis and a fluorescent nucleic acid staining dye for gel visualisation. The master mix contains all required components for PCR, except specific primers. 1mL is suitable for 100 reactions of 20µL.

Quality Control

Functionally tested in PCR.

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Storage

Red MasterMix Fluoro (2X) with gel staining should be stored immediately upon receipt at -20°C. As it is light sensitive, it should be stored protected from light.
When stored under these conditions and handled correctly, these products can be kept up to 1 year without showing any reduction in performance. Besides this, the Red MasterMix Fluoro (2X) could be stored at RT (+15°C to +30°C) up to 3 months and at +4°C up to 6 months without showing any reduction in performance.

Product Use Limitations

Red Mastermix Fluoro (2X) with gel staining dye is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

Related products

Product	Cat#
Red MasterMix Fluoro (2x) with gel staining dye	M3236.0100
GenLadder 100 bp Plus with gel staining dye	M3237.0050
DNA Loading buffer I Fluoro (6x)	M3323.0001
pBLooK™ LED Transilluminator	M3234.0001

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PCR Protocol Part

Protocol using Red MasterMix Fluoro (2X) with gel staining dye

This protocol serves as a guideline for PCR amplification. Optimal cycling conditions such as incubation times, temperatures, and amount of template DNA depend on the DNA target (GC-content, size, quantity, purity, etc.), primers, buffer composition, $MgCl_2$-concentration and enzyme concentration and must be determined individually. We recommend starting with the basic protocol described below and subsequently optimize the annealing temperature, incubation times and cycling numbers.

Important notes before getting started

Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Prepare a master mix according to Table 1.**
   To minimize the risk of contamination or reagent loss and to improve pipetting accuracy, we recommend preparing a master mix for multiple samples containing all the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment.

2. **Mix your master mix with all components except the template DNA**. Keep the master mix on ice.

Table 1: PCR reaction components (**20µL PCR reaction**)

Components	Quantities	Final Concentration
Red MasterMix Fluoro (2X)	10µL	1X
Forward primer (5-10µM)	variable	0.1-0.2µM
Reverse primer (5-10µM)	variable	0.1-0.2µM
Template DNA	variable	4pg-500ng*
PCR grade water	up to 20µL	

For smaller/larger reaction volumes, scale it down/up proportionally.
* If DNA concentration is less than 4pg, it may cause a migratory shift when performing gel electrophoresis. To remedy this, we recommend removing the fluorescent dye prior to post-staining with our DNA Loading buffer I Fluoro (6x) (M3323) again for restoring the DNA molecular weight in the original position. To remove the fluorescent dye, immerse the PCR product containing the fluorescent dye into 100mM NaCl and add 2.5 volumes of absolute or 95% ethanol. Incubate on ice for 20 min and centrifuge the mixture at 4°C for at least 10 minutes. Then remove the suspension of ethanol and wash the pellet with 1mL of 70% ethanol. Dry the residual ethanol and resuspend the dsDNA in the TE buffer.

3. **Mix the master mix thoroughly** and dispense appropriate volumes into PCR tubes.
   Mix gently, for example, by pipetting the master mix up and down. Do not vortex as this may harm the enzyme. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.

4. **Add template DNA** (or PCR grade water in case of the control) to the individual tubes containing the master mix.

5. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

6. **Place PCR tubes in the thermal cycler** and start program.

7. **Analyse PCR products by agarose gel electrophoresis**. Samples can be loaded directly onto the gel without the need of adding an additional loading buffer.

8. **After PCR reaction, direct load onto the gel** for gel electrophoresis is possible.

9. **Use a UV or blue-light transilluminator** to visualise the bands.

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Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	2-5 min	94°C	
Denaturation:	20-40 sec	94°C	
Annealing:	1 min	Ta*	Ta* should be 2°C below the Tm** value of the primer with the lowest Tm
Extension:	2 min	72°C	
Number of Cycles	25 - 35		
Final extension	5 min	72°C	

* Ta is the annealing temperature

** Tm is the melting temperature of a primer which is defined as the temperature at which 50% of the primer bind to the complementary sequence of the target DNA.
Please note: By increasing Ta above Tm, this percentage decreases, however, primers will still anneal up to a certain degree and initiate extension. Therefore, PCR would still work with a Ta of several degrees higher than the Tm but with a dramatically reduced efficiency. Hence, we recommend optimizing the Ta by performing a temperature gradient (e.g., starting at the lowest Tm or a few degrees below and increasing with 2°C increments).

Recommendations

- **Annealing temperature (Ta)**
  Optimization of the annealing temperature is important to guarantee an optimal amplification. We recommend performing a temperature gradient and using primers with a Tm >60°C.
- **Primer concentration**
  The final primer concentration should be in the range of 0.1µM to 0.2µM.

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