GENAXXON bioscience

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# Green DNA Dye (100X)

fluorescence dye for real-time PCR

| Product | Cat# | Package size |
|---|---|---|
| Green DNA Dye (100X) | M3217.0300 | 3 x 100µL |

## General description

Green DNA Dye is a sensitive fluorescent dye for detection of dsDNA in real-time PCR and other applications. When fluorescent dye is free in solution, it emits a very low fluorescent signal. As soon as the dye binds to the double-stranded DNA, the signal increases significantly (thousand fold), which makes the fluorescent signal of the dye directly proportional to the amount of amplified dsDNA.

If the dye is used as stain in agarose or polyacrylamide gels Green DNA Dye is about 4 times more sensitive compared to ethidium bromide.

## Properties and application

Green DNA Dye is delivered as 100-time solution in DMSO. The concentration of Green DNA Dye is traditionally given as a dilution factor where the 1X dilution is used for staining of DNA gels. For PCR, a 0.2X dilution is the starting dilution for optimization.

Each lot of Green DNA Dye is functionally tested in real-time PCR.

## Using Green DNA Dye in qPCR / Dilution for real-time PCR

The Genaxxon Green DNA Dye is supplied as a 100X solution in DMSO.

Green DNA Dye is suitable for real-time PCR with a suggested concentration for short targets of 0.2X (short targets: 50 – 400bp). Dilute delivered 100-time stock solution with nuclease-free PCR grade water by 1:10 (pre-dilution) and add 0.2µL of this solution to a 20µL final volume (PCR reaction). The 10-time working solution has to be kept at -20°C and has to be renewed every 2-3 weeks latest.

Prepare fresh dilutions (working solutions) of the Green DNA Dye prior to reaction set-up and keep all tubes containing Green DNA Dye protected from light. Make initial dilutions of the Green DNA Dye using nuclease-free PCR-grade water. For short amplicons (50 – 400bp) the optimal concentration of Green DNA Dye is usually around 0.2X in the final PCR reaction (see Optimization of Green DNA Dye concentration).

Ready to use master mixes for real-time PCR including Green DNA Dye are also available. See section Related Products.

## Using Green DNA Dye as staining dye in agarose or polyacrylamide gels

Perform electrophoresis on an agarose or nondenaturing polyacrylamide gel. TBE (89mM Tris base, 89mM boric acid, 1mM EDTA, pH8) and TAE (40mM Trisacetate, 1mM EDTA, pH8) buffers are both compatible with Green DNA Dye staining.

Dilute the stock solution of Green DNA Dye 1:100 (stain may be diluted in TE (10mM Tris/HCl, 1mM EDTA, pH8.0), TBE, or TAE buffer. Dilution of 1:100 means e.g. using 500µL for a 50mL gel (adding 500µL of Green Dye DNA 100X solution to 50mL of melted agarose in TAE or TBE buffer).

Staining with Green DNA Dye is pH sensitive. For optimal sensitivity, verify that the pH of the staining solution at the temperature used for staining is between 7.5 and 8.0 (preferably pH 8.0).

Staining solutions prepared in water are less stable than those prepared in buffer and must be used within 24 hours to ensure maximal staining sensitivity. In addition, staining solutions prepared in buffers with pH below about 7.5 or above 8.0 are less stable and show reduced staining efficacy.

Cover the gel with staining solution and incubate at room temperature for 10-40 minutes. Use a plastic container, such as the top of a pipet-tip box. Do not use a glass container, as it will adsorb much of the dye in the staining solution.

Protect the staining container from light by covering it with aluminum foil or placing it in the dark. Agitate the gel gently at room temperature.

Staining time will vary depending on the thick-ness of the gel and the percentage of agarose or polyacrylamide. **No destaining is required!**

The staining solution may be stored in the dark (preferably refrigerated) for a week or more and reused up to four times.

We recommend storing aqueous stain solutions in plastic rather than glass, as the stain may adsorb to glass surfaces.



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### Visualisation of stained DNA by UV or blue-light transillumination

DNA stained with Green DNA Dye can be readily visualized using a UV or blue-light sources.

Epi-illumination with 254 nm will give a higher sensitivity than 300 nm transillumination.

It is important to clean the surface of the transilluminator after each use with deionized water and a soft cloth. Otherwise, Green DNA Dye as other fluorescent dyes, such as GelRed and ethidium bromide, will accumulate on the glass surface and cause a high background fluorescence.

### Storage and Stability

The unopened product is stable for 1 year at -20°C and protected from light.
Note: The fluorescence signal may decrease slightly over time. Therefore, it is recommended to re-evaluate the applied concentration of Green DNA Dye after approximately 6 months.
Note: Green DNA Dye is not stable when diluted in water!

### Optimization of Green DNA Dye concentration

The fluorescence signal of Green DNA Dye may vary slightly from batch to batch and may decrease over time. The optimal concentration of Green DNA Dye in real-time PCR should be evaluated for each new batch. Too low concentrations of Green DNA Dye lead to a low fluorescent signal which gives a bad signal to background ratio. Too high concentrations of Green DNA Dye lead to the inhibition of DNA polymerases and result in a high Ct value and low efficiency. The degree of inhibition depends not only on the amount of Green DNA Dye but also on the buffer composition used.

To find the optimal concentration of Green DNA Dye or for re-evaluation, a real-time PCR is run with various concentrations of Green DNA Dye. Some batches might need less than 0.2X, others including older batches might need more. The optimal concentration is the one with the highest possible fluorescence level combined with the lowest Ct value and an efficiency of 100 +/-10%.

### 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).

### Related real-time PCR products

| Product | Cat# | Package size |
|---|---|---|
| GreenMasterMix (2X) No ROX | M3023.0100 | 200 x 25µL PCR reactions |
| GreenMasterMix (2X) No ROX | M3023.0500 | 1000 x 25µL PCR reactions |
| GreenMasterMix (2X) Low ROX | M3011.0100 | 200 x 25µL PCR reactions |
| GreenMasterMix (2X) Low ROX | M3011.0500 | 1000 x 25µL PCR reactions |
| GreenMasterMix (2X) High ROX | M3052.0100 | 200 x 25µL PCR reactions |
| GreenMasterMix (2X) High ROX | M3052.0500 | 1000 x 25µL PCR reactions |
| ProbeMasterMix (2X) No ROX | M3034.0100 | 200 x 25µL PCR reactions |
| ProbeMasterMix (2X) No ROX | M3045.0500 | 1000 x 25µL PCR reactions |
| GreenMasterMix (2X) Low ROX | M3031.0100 | 200 x 25µL PCR reactions |
| GreenMasterMix (2X) Low ROX | M3031.0500 | 1000 x 25µL PCR reactions |
| GreenMasterMix (2X) High ROX | M3010.0100 | 200 x 25µL PCR reactions |
| GreenMasterMix (2X) High ROX | M3010.0100 | 1000 x 25µL PCR reactions |
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| SuperHotstart Taq Polymerase | M3307.0250 | 250 units |
| SuperHotstart Taq Polymerase | M3307.1000 | 1000 units |
| | | |
| dNTP-Set 100mM | M3015.4100 | 4 x 1mL |
| dNTP-Set 100mM | M3015.4500 | 4 x 5mL |



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