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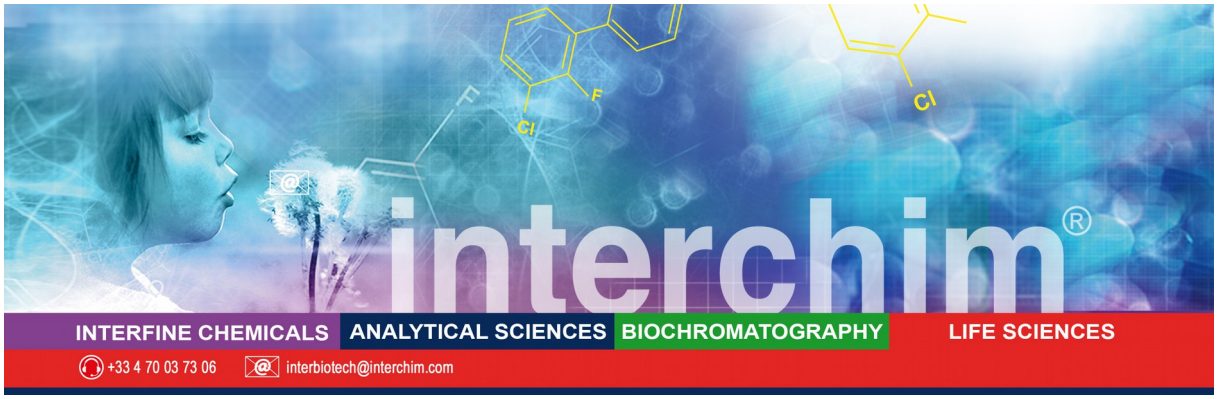


# EvaGreen® dsDNA Reagent

*A smart quantitative dye that detects dsDNA via a novel release-on-demand binding mechanism for Real-Time PCR, High Resolution Melt (HRM) and DNA quantification in solution*

## Product Information

| **Product name**<br>cat.number | Comments |
|---|---|
| **EvaGreen® 20X** (in 1X PBS pH7.3, with< 2% DMSO)<br>**BI1790**, 5x1 ml<br>(2000 qPCR, 1000 DNA quantifications) | - $\lambda_{exc.}$/ $\lambda_{em.}$(DNA bound): 500 / 530 nm<br>- $\lambda_{abs.}$(without DNA): 471 nm<br>- Orange solution at room temperature |
| **EvaGreen® qPCR 2X Basic Mix** (for non-HotStart Taq)<br>**BZ2180**, 3x1.7 ml | The Basic Mix contains everything you need to run a qPCR except for the Taq enzyme. |
| **EvaGreen® qPCR 2X Basic Mix (for HotStart Taq)**<br>**CK7270**, 3x1.7 ml | The Basic Mix contains everything you need to run a qPCR except for the HotStart Taq enzyme. |
| **EvaGreen® 20 000X in DMSO**<br>**CA6770**, 1 ml<br>(400 000 qPCR, 200 000 DNA quantifications) | Concentrated solution (25 mM) |

**Storage:** 4°C or below to prevent mold formation. Protect from light.

## Introduction

EvaGreen® dye is a green fluorescent nucleic acid dye with features had make the dye useful for several applications including qPCR, highresolution DNA melt curve analysis (HRM), real-time monitoring of thermophilic helicase-dependent amplification (tHDA), routine solution DNA quantification and capillary gel electrophoresis. The DNA-bound dye has excitation and emission spectra very close to those of fluorescein (FAM), making the dye readily compatible with instruments equipped with the 488 nm argon laser or any visible light excitation with wavelength in the region. EvaGreen® dye is extremely stable both thermally and hydrolytically (**Figure below**), providing convenience during routine handling. The dye is essentially nonfluorescent by itself, but becomes highly fluorescent upon binding to dsDNA.

EvaGreen® dye is nonmutagenic and noncytotoxic by being completely impermeable to cell membranes, unlike Green competitor, which enters cell rapidly and is known to be a powerful mutation-enhancer (Ohta, et el. *Mutat. Res.* **492**, 91(2001)).

The unique properties of EvaGreen® dye have made it particularly useful in quantitative real-time PCR (qPCR) application. Compared with the widely used Green competitor, EvaGreen® dye is generally less inhibitory toward PCR and less likely to cause nonspecific amplification. As a result, EvaGreen® dye can be used at a much

higher dye concentration than Green competitor, resulting in more robust PCR signal. More significantly, the higher EvaGreen® concentration permitted for qPCR eliminates so-called "dye redistribution" problem, which can occur with Green competitor during post PCR DNA melt curve analysis. Dye redistribution problem may make Green competitor unreliable for regular DNA melt curve analysis (Giglio, et al. Nucleic Acid Res. **31**(22), e136(2003)) and also unsuitable for HRM (Wittwer, et al. Clin. Chem. **49**(6), 853(2003)). On the other hand, EvaGreen® dye is suitable for both qPCR and HRM, yielding robust and reproducible results.

In addition to qPCR application, EvaGreen may be used for general **dsDNA quantification** in solutions. The dye, however, is not suitable for staining DNA in live cells because it is not cell membrane-permeable.

Ames test performed by an independent lab, Litron Laboratories (Rochester, NY), showed that EvaGreen® dye is nonmutagenic as well as noncytotoxic. EvaGreen® dye appears to be completely cell membrane impermeable, which may be a key factor responsible for the observed low toxicity (See EvaGreen safety report). On the other hand, Green competitor is known to be a powerful mutation enhancer, possibly by inhibiting the natural DNA repairing mechanism in cells (Ohta, et al. *Mutat. Res.* **492**, 91(2001)). The toxicity of Green competitor may be associated with its ability to enter cells rapidly.

Since these toxicity tests were not performed on human, we still advise that researchers exercise precautions when handling the dye or any other DNA-binding molecules by wearing protective gears.

The combination of sensitivity, safety and stability makes EvaGreen® an excellent choice for many nucleic acid detection applications.

## Directions for use

### Handling and Storage

EvaGreen® dye is generally very stable. We recommend EvaGreen® 20X solution be stored at 4°C or below to prevent mold formation. The expected shelf-life under the recommended condition should be at least 12 month from the date of receipt. When taking the dye solution out of the freezer, vortex the solution for a few seconds in case of dye adsorption on the container wall during storage.

### Guidelines for use

Protocol for qPCR for non-HotStart Taq

EvaGreen 20X solution is specifically formulated for qPCR use. PCR reaction can be monitored using your existing optical setting for Green competitor or FAM on any commercial real-time PCR cycler. The qPCR protocol provided below is for PCR using **regular non-hot-start Taq**. Use of a hot-start Taq may require some adjustment of PCR buffer composition in terms of ionic strength and pH to best take the advantage of EvaGreen™ dye. For example, chemically-modified Taq, such as AmpliTaq Gold, may prefer a downward-adjustment of KCl concentration (to as low as 0.0 mM) and an upward-adjustment of Tris concentration (to as high as 50 mM). In addition, a water soluble solvent such as DMSO or glycerol has traditionally been added to stabilize a master mix. These components plus the pH may need to be optimized depending on the nature of your enzyme. Nevertheless, if you use a regular non-hot-start Taq and follow the protocol provided below, you should expect to see superior performance from EvaGreen® dye over that from Green competitor. Because the optical settings vary slightly from instrument to instrument and the wavelengths of EvaGreen® dye are slightly longer than those of Green competitor, Ct value may differ slightly by +1 or –1 when compared with Green competitor side-by-side. However, regardless of which cycler you use, the fluorescence signals with EvaGreen® dye for both qPCR and melt curve analysis should be significantly stronger than those with Green competitor.

Recommended qPCR Procedure for 50 µL-sized reactions:

1) Set up PCR reaction as follows[1]:
   - 5 µL of 10x polymerase buffer without Magnesium[2]
   - 2.5 µL of 50mM $MgCl_2$ [3]
   - 5 µL each of 2 mM dNTP
   - 2.5 µL of 20X EvaGreen® [4]
   - 1-5 units of Taq DNA polymerase or a Hot Start Taq DNA Polymerase [5]
   - 0.1-1 µM each of primers (final concentrations)

Add Di-$H_2O$ to make a final volume of 50 µL.

2) Perform real-time PCR reaction on a thermocycling fluorometer and record the fluorescence signal at the annealing or extension step.

*[1] For iCycler users, you do not need to add FAM to your PCR mix since EvaGreen has a slight background fluorescence that provides an adequate and stable baseline level fluorescence for well calibration.*

*When using ABI Sequence Detection Systems, make sure to select NONE for the passive reference under the tab WELL INSPECTOR.*

*BSA may be required if the reaction is run on a Roche LightCycler. A final BSA concentration of 0.5mg/mL may be sufficient. With Green competitor, addition of a protein such as BSA results in a fluorescence increase, which provides a background signal that triggers the start of a LightCycler. Since EvaGreen dye is less sensitive to proteins, you may need to adjust the instrument setting (for background fluorescence) so that the instrument will start.*

*[2] For chemically-modified Taq, it may be necessary to downward adjust KCl concentration and upward adjust Tris concentration.*

*[3] The optimal $Mg^{2+}$ concentration for PCR with EvaGreen should be 2.5 mM.*

*[4] Before pipetting, warm up the 20X solution to room temperature and thoroughly mix the solution by vortexing. EvaGreen is highly stable. However, dye adsorption onto container wall may occur during storage at low temperature over a long period of time. Should it occur, vortexing the vial for a few seconds should alleviate the problem.*

*[5] For best results, a hot-start enzyme should be used. However, buffer condition may need to be adjusted accordingly to best take the advantage of the dye.*

Protocol for qPCR for HotStart Taq

EvaGreen® qPCR 2x Basic Mix HS is formulated for hotstart *Taq*. It contains all the necessary components for qPCR except for hotstart *Taq* polymerase, primers and a template. It is compatible with all known commercial qPCR instruments. PCR reactions can be monitored using your existing instrument setting for Green competitor or FAM. Because optical settings may vary from instrument to instrument and the wavelengths of EvaGreen® dye are slightly longer than those of Green competitor, Ct value may also differ by +1 or –1 when compared with Green competitor side-byside. However, regardless of the type of cycler you use, the fluorescence signal with EvaGreen® in general should be significantly stronger than that with Green competitor. For example, on ABI 7900, reactions carried out with 10µL of 1x EvaGreen® qPCR mix can be expected to generate more fluorescence than those carried out with 50 µL of Power Green competitor mix.
EvaGreen® qPCR 2x Basic Mix HS has been tested for use with hotstart DNA polymerases from the following sources:

| **DNA Polymerase** | **Manufacturer** |
|---|---|
| AmpliTaq Gold | Applied Biosystems |
| Hot Start Taq | Fermentas |
| Platinum Taq | Invitrogen |
| AccuPrime Taq with High Fidelity | Invitrogen |
| Platinium Tfi | Invitrogen |

**Recommended qPCR Procedure for 20 µL-sized reactions:**
1) Set up PCR reaction by adding to each well[1]:
- 10 µL of 2x EvaGreen® qPCR Basic Mix
- 2.5-10 units of a HotStart Taq DNA polymerase
- 0.1-0.5 µM (final concentration) each of primers
- Template
- Di-water to a total volume of 20 µL/reaction.

2) Carry out the reaction and detection using one of the following protocols:

A. Universal Protocol[2]
Set the cycling parameters as follows when AmpliTaq Gold is used:
- 95 °C – 10 minutes
- 45 cycles of 95 °C – 15 seconds

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60 °C - 60 seconds
Record fluorescence in FAM channel.

B. Fast Protocol[2]

Set the cycling parameters as follows when Fermentas HotStart Taq, Platinum Taq, AccuPrime Taq, or Platinum Tfi is used:

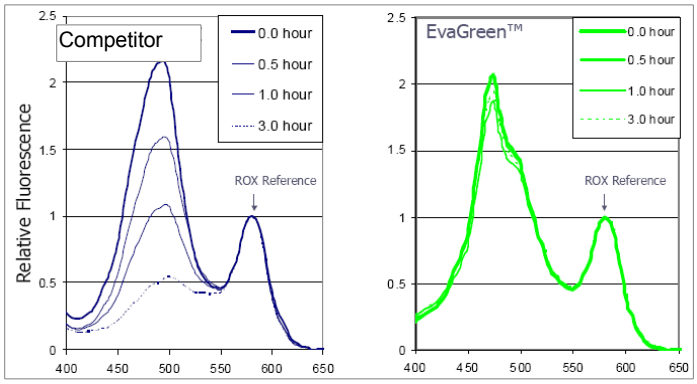
96 °C – 2 minutes
45 cycles of 96 °C – 5 seconds
60 °C -5 seconds
60 °C – 70 °C -25 seconds
Record fluorescence in FAM channel.

*1 For iCycler users, you do not need to add FAM to your PCR mix since EvaGreen has a slight background fluorescence that provides an adequate and stable baseline level fluorescence.*

*BSA may be required if the reaction is run in glass capillaries on a Roche LightCycler. A final BSA concentration of 0.5mg/mL may be sufficient.*

*2 The Basic Mix does not contain a reference dye (such as ROX), which may be required on some PCR instruments. When using ABI Sequence Detection Systems, make sure to select NONE for the passive reference under the tab WELL INSPECTOR.*

**Stability Comparison between EvaGreen™ and Green competitor in Tris pH 9 at 99°C**




Wavelength (nm)

**Guidelines for use - <u>Protocol for dsDNA quantification in solution</u>**

EvaGreen® solution is useful to quantitate DNA especially in complex mixtures compared with alternative methods:
-measuring nucleic acid concentration by absorbance at 260 nm (A260) is very popular, but presents disadvantages, starting with 1/the non distinction of DNA and nucleotides and single-stranded nucleic acids as well as RNA that contribute to the signal, 2/interferences of compounds found in nucleic acid preparations, 3/the relative insensitivity of the measurement (5 µg/mL dsDNA solution for an A260 of 0.1).
-methods using Hoechst (bisbenzimide) dyes that improve selectivity for dsDNA, smaller interferences from proteins, and sensitivity

- Reagent Preparation

The EvaGreen® dsDNA Reagent is supplied as 20X and 20 000X concentrated dye solutions.
With the 20 000X concentrated dye solution, prepare an 20X working solution of EvaGreen™ dsDNA Reagent by making a 1:1000 dilution with 1X PBS buffer, pH7.3.
Protect the working solution from light by covering it with foil or placing it in the dark.

- DNA Standards and sample preparation

Prepare a 20 µg/mL stock solution of dsDNA in TE, then dilutions for the standard curve (see table 1).



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Determine the DNA concentration on the basis of absorbance at 260 nm (A260) in a cuvette with a 1-cm path length; an A260 of 0.4 corresponds to 20µg/mL dsDNA solution. Calf thymus DNA is commonly used for a standard curve, although any purified dsDNA preparation may be used. It is preferable to prepare the standard curve with DNA similar to the type being assayed; long or short linear DNA fragments for quantitating similar-sized restriction fragments; plasmid for quantitating plasmid DNA. However, most linear dsDNA molecules have been found to yield approximately equivalent signals, regardless of fragment length.
To serve as an effective control, the dsDNA solution used to prepare the standard curve should be treated the same way as the experimental samples and should contain similar levels of such compounds.
Samples should be diluted with TE if required (if to much concentrated, interfering substances)

**Table 1:** Standards and sample preparation for 50 µL-sized reactions:

| Tube | dsDNA solution (µL) 20 µg/ml | dsDNA solution (µL) 2 µg/ml | Sample (µL) | TE Buffer (µL) | Final dsDNA (ng) | EvaGreen 20X (µL) |
|---|---|---|---|---|---|---|
| 0 (Blk) | - | - | - | 50 | 0 | 5µL |
| 1 | 50 | - | - | 0 | 1000 | 5µL |
| 2 | 25 | - | - | 25 | 500 | 5µL |
| 3 | 5 | - | - | 45 | 100 | 5µL |
| 4 | - | 40 | - | 10 | 80 | 5µL |
| 5 | - | 30 | - | 20 | 60 | 5µL |
| 6 | - | 20 | - | 30 | 40 | 5µL |
| 7 | - | 10 | - | 40 | 20 | 5µL |
| Sample | - | - | Vx | 50-Vx | unknown | 5µL |

- Sample analysis: Recommended dsDNA Assay procedure for 50 µL-sized samples.

1. Add 5 µL of 20X EvaGreen to 50 µL of DNA samples or dsDNA standards.

2. After 2 minutes of incubation, measure the sample fluorescence in the fluorometer using a fluorescein filter ($\lambda_{exc./em.}$: 488/520 nm)

*An instrument calibration may be required: Insert the most fluorescent standard (20µg/mL) first and adjust the fluorometer sensitivity factor, to accommodate the lower fluorescence signals. Measure the fluorescence of the remaining samples. Plot a standard curve after subtracting the reagent blank fluorescence value.*

3. Calculate the DNA concentration from the standard curve:
-Subtract the reagent blank fluorescence value.
-Determine the DNA concentration of the sample from the standard curve generated in DNA Standard Curve.

**Disposal**

EvaGreen® solution may be disposed of using one of the following methods:

1) Add 25~50 mL bleach (regular household bleach) to each gallon (~4L) of the waste solution containing the dye and let the mixture react for at least 8 hours before pouring the solution to a sink
2) Pour each 10 liters of EvaGreen® waste solution through ~1g of activated charcoal. The filtrate may directly go to the drain while the charcoal may be treated as regular solid waste.

## References

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## Related products

- FAST Plus EvaGreen qPCR MASTER MIX, GV9900
- Fast EvaGreen master mix for qPCR and HRM, DV7220
- Lambda DNA, UP947860
- FP-M1319A
- GelRed, nucleic acid gel stain, BY1740
- GelGreen, nucleic acid gel stain, BY1750
- dNTP Set 1, 100mM, UP968640
- UptiTherm polymerase, UPS53921
- HotStart Taq DNA Polymerase, Q6563A
- Agarose, regular uses, 31272L

## Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06



* EvaGreen and its uses are covered by pending US and international patents.
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