Product Manual

CYGREEN® Nucleic Acid Dye

Catalog #: ENZ-GEN105-0100

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Product Manual

TABLE OF CONTENTS

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

DESCRIPTION

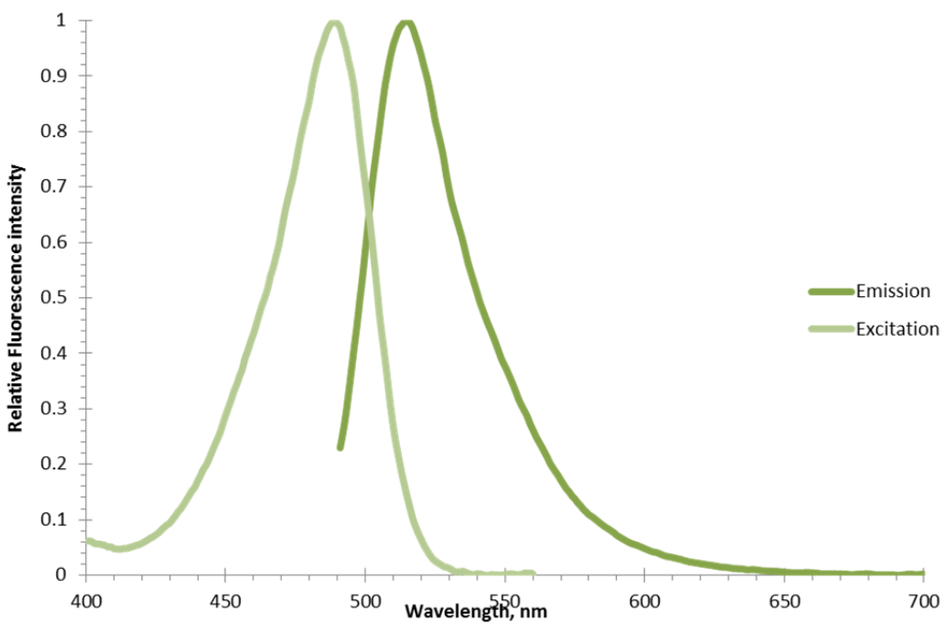
CYGREEN® Nucleic Acid Dye is a DNA intercalating agent that is used to stain DNA. The DNA-dye complex emits a fluorescence spectra that makes it suitable for qPCR and gel staining applications.

INTRODUCTION

In real-time or quantitative PCR (qPCR), the amplification of amplicon product can be monitored in real-time as the polymerase chain reaction occurs as a fluorescence intensity. The fluorescent signal generated during the exponential phase of the PCR reaction is proportional to the amount of amplicon produced and can be used to quantify the amount of template DNA used in the reaction.

Using an intercalating DNA dye is the most popular non-probe based qPCR methodology. It is the most cost-effective and convenient type of chemistry for qPCR. Enzo's CYGREEN® Nucleic Acid Dye shares spectral properties with common dyes used in qPCR and is intended as a replacement.

In addition, CYGREEN® Nucleic Acid Dye has been validated for use in gel staining.



Excitation and Emission Spectra of CYGREEN® Nucleic Acid Dye bound to DNA. CYGREEN® Nucleic Acid Dye shares spectral properties with other commonly used qPCR dyes, allowing detection in the FAM (or similar) channel.

SAFETY WARNINGS & PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- Since CYGREEN® binds to nucleic acids, it should be treated as a possible mutagen and should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

MATERIALS SUPPLIED

CYGREEN® Nucleic Acid Dye

100µl of a 10,000x concentration.

STORAGE

CYGREEN® Nucleic Acid Dye is stable for 12 months. It should be stored at or below -20°C

OTHER MATERIALS REQUIRED

For qPCR Applications:

1. Nuclease-free water
2. AMPIGENE® dNTP Mix (Prod. No. ENZ-NUC102)
3. AMPIGENE® HS Taq polymerase (Prod. No. ENZ-PRT101)
4. Primers
5. DNA Template
6. Thermal Cycler

For Gel staining:

7. TBE or TAE Buffer
8. Agarose
9. Electrophoresis Apparatus

PROCEDURAL NOTES

AMPIGENE® HS Taq polymerase (Prod. No. ENZ-PRT101) is provided with a 5x AMPIGENE® reaction buffer that contains dNTPs. However, additional dNTPs can be supplemented or alternative reaction buffers can be used. Adjust the volume of the nuclease-free water to accommodate other reaction component changes, bringing the reaction volume to the same final suggested volume.

REAGENT PREPARATION

Upon receipt of reagent, it is recommended to make a 100x stock solution and to freeze aliquots at -20°C. CYGREEN® Nucleic Acid Dye can be diluted in water. Further dilute as necessary depending on assay procedure.

The final recommended concentration in a qPCR reaction or gel staining is 1x. A working 10x solution should be made fresh daily.

QPCR SUGGESTED PROCEDURE

The following is a guideline for qPCR set-up. CYGREEN® Nucleic Acid Dye can be substituted for commonly used qPCR DNA dyes. **Optimization of qPCR reaction conditions is recommended.**

1. Prepare master mix as follows:
    - 6.0 µL Nuclease-free water
    - 5.0 µL 5x AMPIGENE® reaction buffer
    - 1.0 µL Primer
    - 0.5 µL AMPIGENE® HS Taq DNA Polymerase
    - 2.5 10x CYGREEN® Nucleic Acid Dye
    - 10 µL Template

      Total 25 µL
2. Perform qPCR on thermal cycler and record the fluorescence signal at the annealing or extension step in the FAM channel (or similar channel).

GEL STAINING SUGGESTED PROCEDURE

The following is a guideline for Gel Staining with CYGREEN® Nucleic Acid Dye. **Optimization of gel electrophoresis and staining is recommended.**

1. Perform electrophoresis on an agarose gel using TBE or TAE Buffer.
2. Dilute desired volume of CYGREEN® Dye with buffer to 1x.
3. Use a plastic container for staining. Cover the gel with staining solution and incubate for ~30 minutes.
   - Mix gel and staining solution gently at room temperature
   - Staining time will vary depending on thickness of the gel and the % agarose.
4. Gel can be imaged with UV or blue-light sources, as well as laser scanning instruments.
5. CYGREEN® Dye can be removed by simple ethanol precipitation.

CYGREEN® GEL CASTING SUGGESTED PROCEDURE

CYGREEN® Nucleic Acid Dye can be added directly to the agarose prior to casting. The following is a guideline for precasting gels with CYGREEN® Nucleic Acid Dye. **Optimization of concentration of CYGREEN® Nucleic Acid Dye to add to gel is recommended.**

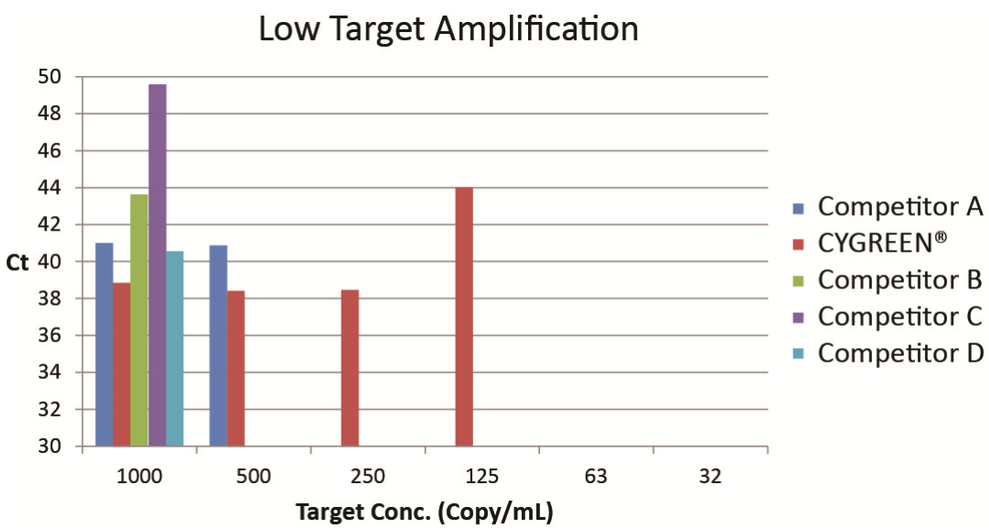
1. Prepare an agarose solution. Cool gel solution 5-10 min at room temp.
2. Add CYGREEN® Nucleic Acid Dye to 1x into cooled agarose gel solution and mix well prior to pouring the gel.
3. Load desired amount of DNA onto gel
4. Perform electrophoresis using TBE or TAE Buffer
5. Gel can be imaged with UV or blue-light sources, as well as laser scanning instruments.

DNA STAINING PRIOR TO GEL ELECTROPHORESIS

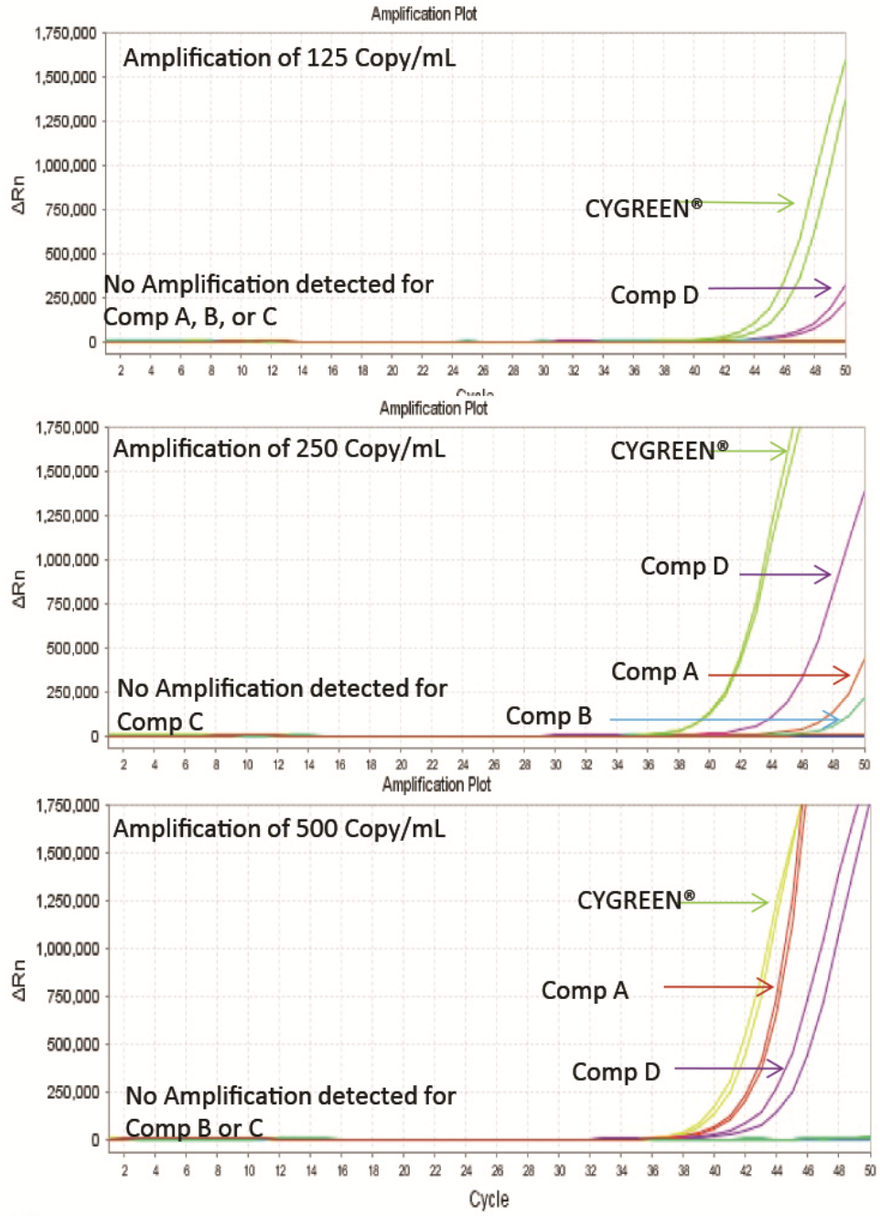
CYGREEN® Nucleic Acid Dye can be added directly to the DNA prior to loading onto the gel. The following is a guideline for staining DNA with CYGREEN® Nucleic Acid Dye. **Optimization of concentration and staining duration is recommended.**

1. Stain DNA with 1x-10x of CYGREEN® Nucleic Acid Dye for 10-15min.
2. Load desired amount of DNA onto gel
3. Perform electrophoresis using TBE or TAE Buffer
4. Gel can be imaged with UV or blue-light sources, as well as laser scanning instruments.

APPENDIX A: QPCR COMPARISON

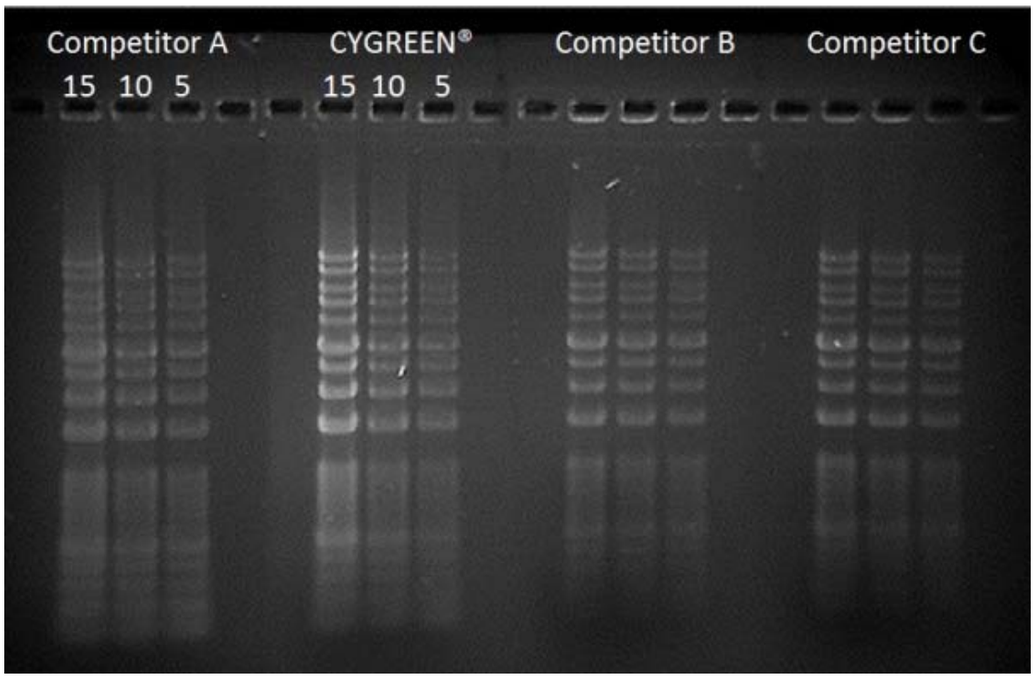


CYGREEN® Nucleic Acid Dye and 4 other Competitor Dyes were used in qPCR reactions with varying target concentrations. CYGREEN® Nucleic Acid Dye has higher sensitivity than Competitor Dyes and was able to detect low target concentrations.



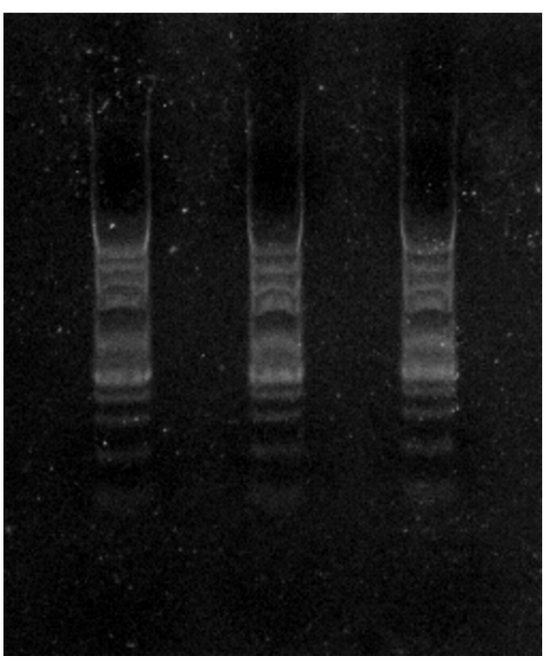
Amplification curves of CYGREEN® Nucleic Acid Dye and 4 other Competitor Dyes used in qPCR reactions with varying target concentrations. CYGREEN® Nucleic Acid Dye has higher sensitivity than Competitor Dyes.

APPENDIX B: GEL STAINING COMPARISON



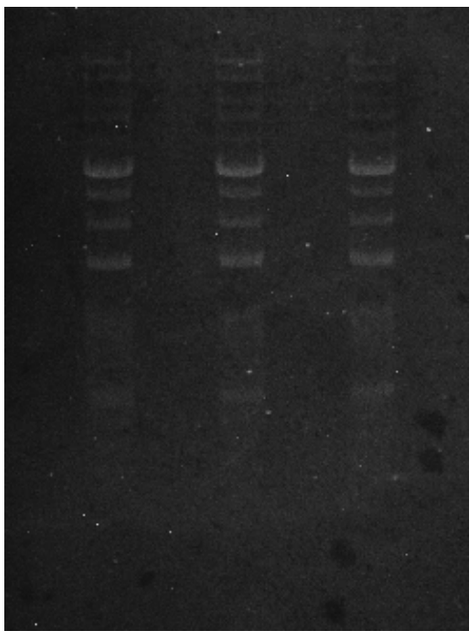
5, 10 and 15µL of AMPIGENE® DNA Ladder 100-10,000 bp (ENZ-GEN104) were loaded onto a 1% Agarose gel in TAE buffer and run 1h at 100v. Gel was cut into sections and stained for 1h with CYGREEN® Dye or with 3 other competitors. CYGREEN® Dye shows bright gel staining equivalent or better than competitors.

APPENDIX C: CYGREEN® GEL CASTING



10µL of CYGREEN® Nucleic Acid Dye was added to 100mL of a 1% Agarose solution. Gel was poured. 15µL of AMPIGENE® DNA Ladder 100-10,000 bp (ENZ-GEN104) was loaded into three lanes of thegel and was run in TAE buffer for 1h at 100v. CYGREEN® Nucleic Acid precast gel shows bright staining.

APPENDIX D: DNA STAINING BEFORE GEL ELECTROPHORESIS



AMPIGENE® DNA Ladder 100-10,000 bp (ENZ-GEN104) was stained with a 1/10 dilution of a 100x solution of CYGREEN® Nucleic Acid Dye (for a 10x final concentration of CYGREEN®Dye)at room temp for 10 min. 15µL of the stained DNA ladder was loaded into each of 3 wells of a 1% agarose gel. Gel was run for 1h at 100V. CYGREEN® Nucleic acid dye prestained DNA can be visualized in a gel.

FAQs

What if the amplitude of the amplification curve is low?

Dye optimization (titration) is recommended with enzyme and buffer system used.

What channel in the thermal cycler should be used?

The FAM channel or SYBR® Green channel should be used for detection.

What should be done if the intensity of DNA bands in the gel is low?

Stain the gel for a longer period or use more dye. Staining time and dye concentration is dependent on gel conditions and dye optimization (titration) is recommended.

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com