

User Manual



Chromofy™
dsDNA binding dye

Version 1.3 — February 2010

For use in quantitative real-time PCR



tatabiocenter

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Background

Chromofy™ is a monomeric cyanine dye developed by TATAA Biocenter for use in real-time PCR applications. The dye has absorbance and emission wavelengths suitable for the FAM/SYBR channel on most common real-time PCR instrumentation and shows a very strong fluorescence increase when bound to dsDNA.

Chromofy™ can be used as an unspecific DNA binding dye for real-time PCR applications or other applications where staining of dsDNA is wanted.

Contents

25 µl 5000x stock slution of Chromofy™ in DMSO. Sufficient for approximately 5000 25 µl qPCR reactions.

Fluorescence data

Maximum absorbance has been determined to 482 nm and maximum emission at 507 nm, when bound to dsDNA. The fluorescence increases approximately 450 times when the dye binds to dsDNA.

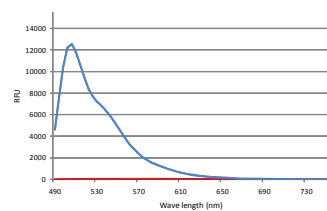


Figure 1: Fluorescence intensity of free Chromofy™ (red) and in presence of dsDNA (blue).

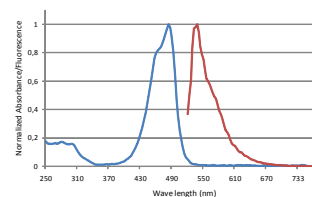


Figure 2: Absorption (blue) and emission (red) spectras of Chromofy™.

Storage

The stock solution can be stored at +4°C for up to 12 months. Once diluted in water the dye can be stored at +4°C for at least 1 month. Repeated freeze-thaw cycles are not recommended.

Additionally required materials and devices

- Real-time PCR instrumentation

The product has been validated on several different instrument platforms.

Note: Using Chromofy™ on certain instruments may need calibration. Most instruments can directly use the FAM/SYBR-channel to monitor the Chromofy signal.

- Mastermix or mastermix components

The dye has been tested using many commercially available mastermixes, including Quantace SensiMix, Roche LightCycler® 480 Probes Master, Fermentas Maxima™ Probe qPCR mastermix, Finnzymes DyNAmo™ Flash Probe kit, Quantace SensiMix HRM™ and PrimerDesign Precision™ HRM MasterMix.

Note: The use of Chromofy™ in certain mastermixes results in a lower and noisier amplification and dissociation curve signal. This has been noticed for example with Applied Biosystems TaqMan® Universal Mastermix.

- Pipettes and tips
- Vortex and centrifuge
- Sample cDNA

Optimal results in real time PCR require high quality DNA samples. Quality of RNA can be tested prior to cDNA synthesis using Agilent 2100 Bioanalyzer or Bio-Rad Experion.

Typical results using Chromofy™

Chromofy™ can be used as a non-specific reporter of dsDNA products and in High-Resolution Melt (HRM) applications.

The end fluorescence level of Chromofy™ is slightly lower than SYBR® Green and similar to that of EvaGreen®. It is recommended to use a high gain setting, if the instrument supports this. The melting temperature of the PCR product is similar to when using SYBR® green. Also, the resulting Ct-values may be shifted towards lower or higher values depending on the mastermix used.

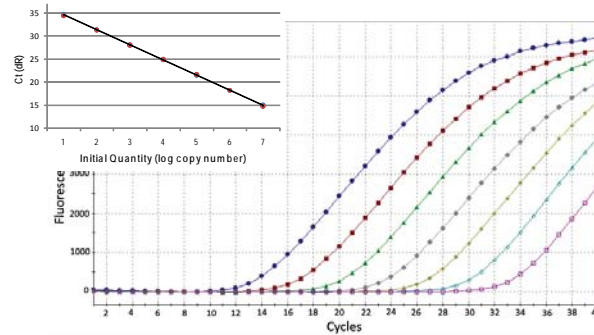


Figure 3: Standard curve of human beta tubulin using Chromofy™ and Roche LightCycler® 480 Probes Master. Efficiency 100 %.

Chromofy™ can also be used for HRM , which is a post PCR analysis where the melting temperature is used to analyze different sample characteristics. HRM is commonly used for genotyping with or without unlabelled probes, and for methylation analysis. Prior to the methylation analysis the DNA is always treated with bisulfite, which converts unmethylated cytosines to uracil that during the PCR will be substituted for thymines. This will result in a methylated sample having higher melting temperature than an unmethylated sample due to higher GC content.

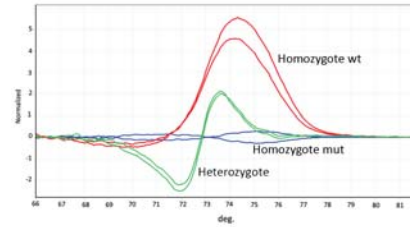
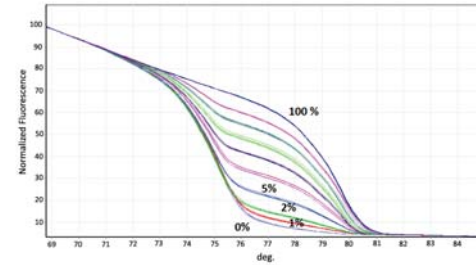


Figure 4: Chromofy™ can easily distinguish between the homozygote wild type (G) and homozygote mutant (A) genotypes of the Factor V Leiden SNP, here using Rotor-Gene 6000.

Figure 5: HRM methylation analysis using Chromofy™ and Rotor-gene 6000. The assay is sensitive down to 1% methylated DNA in unmethylated background, each control in tetraplicates.



Instructions for use

25 µl of stock solution is supplied at 5000x in DMSO and is stable for up to 12 months at 4°C.

Before use, dilute the stock 1:100 to a working solution of 50x by adding 2 µl of the stock solution to 198 µl water. This can be scaled up for larger volumes. A solution of 50x is stable for more than 1 month at 4°C. Typical concentration in real-time PCR reactions is 1-2x, optimal concentration may vary depending on the mastermix. Detection should be done on the FAM/SYBR channel. The dye can be used with most commercially available real-time PCR reagents. The quality of signal may depend on the chemistry used and on the filter settings on the real-time PCR platform. If the instrument used has variable gain settings, Chromofy™ should generally be used at a high gain setting.

Safety

As Chromofy™ binds to DNA it is a potential mutagen and should be treated as such.

Chromofy and Biomark

Chromofy is currently the best available DNA binding dye alternative for the Biomark 48.48 and 96.96 dynamic arrays from Fluidigm. It is compatible with AmpliTaq® Gold 360 from Applied Biosystems and is recommended for use at a 6x final concentration. The Biomark chips have separate wells for loading primers and samples and the dye should be loaded together with the samples.

Troubleshooting

• I do not get any amplification/signal?

The instrument may not have been programmed correctly. Make sure the Chromofy™ dye is monitored in the appropriate channel and that the instrument is calibrated if necessary. If you run the samples on a gel and get visible bands you know that the template has been amplified and the problem lies in the detection or vice versa.

• I get a very noisy amplification and dissociation curve

The quality of signal may depend on the chemistry used and on the filter settings on the real-time PCR platform. If the instrument used has variable gain setting, try to increase the gain. Also make sure that if the instrument requires calibration that this has been performed. Changing the mastermix can also improve the signal.

• My negative controls give a positive amplification?

Since Chromofy is a dsDNA binding dye it will also bind to non-specific products such as primer dimers. Amplification of non-specific products will lead to an amplification signal. The amplified product can be evaluated by doing dissociation curve analysis.

• My samples have same/higher C_q-value than my negative controls?

This indicates that you have added too little DNA. Add more DNA and try again. The DNA may be of low quality. Check the quality of the RNA before doing cDNA synthesis.

• My replicates are not very tight?

With good quality DNA and good pipetting technique, very high reproducibility is possible. Low amounts of DNA can lead to higher variation. Also, low quality DNA can lead to big differences between replicates.

Contact

For more information about the product and other products available from TATAA Biocenter, please contact us on info@tataa.com or visit our website www.tataa.com.

License information

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes. For information on purchasing a license to this product for purposes other than research, contact TATAA Biocenter AB, Odinsgatan 28, S-41103 Gothenburg, Sweden, Phone: +46 31 761 57 00, Fax: +46 31 152890, Email: info@tataa.com

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Other products from TATAA

Human Endogenous Control Gene Panel

A panel containing primer sets for 12 commonly used human reference genes. A perfect product for finding the most optimal reference gene for your samples. GenEx Standard software is also included in the kit.

Mouse Endogenous Control Gene Panel

A panel containing primer sets for 12 commonly used mouse reference genes. A perfect product for finding the most optimal reference gene for your samples. GenEx Standard software is also included in the kit.

GenEx software

A software for gene expression analysis. GenEx provides the appropriate tools to analyze real-time PCR gene expression data and to extract valuable information from the measurements.

TrulyStem - for Human Embryonic Stem Cell Characterization

For easy monitoring of the differentiation state of stem cells using real-time PCR. The kit contains primer sets for 13 well established stem cell differentiation markers and positive control cDNA from differentiated and non-differentiated stem cells.

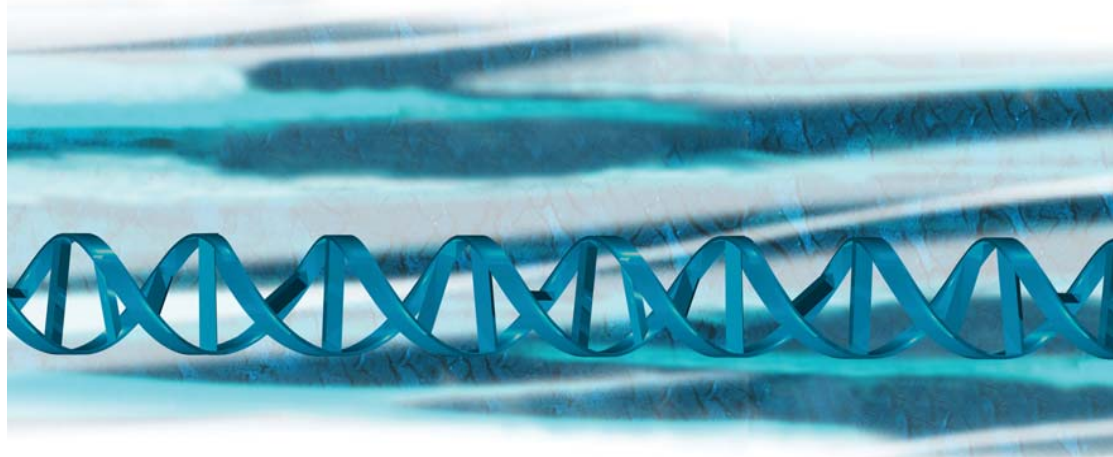
CelluLyser - for rapid and easy lysis and cDNA synthesis

The CelluLyser Lysis and cDNA Synthesis Kit enables you to generate cDNA from small samples with minimal losses and hands-on time, allowing even a single cell to be analysed. A rapid and sensitive lysis is followed by reverse transcription without the need of purification.

VisiBlue qPCR mix colorant

The VisiBlue qPCR mix colorant enables you to quickly color your favourite qPCR mastermix to easily visualize where the reagent has been added to your plates and tubes. VisiBlue is very easy to use by a simple addition to your mastermix.

Express your genius



TATAA Biocenter, with offices in Gothenburg, San Francisco and Prague is the leading provider of real-time PCR services and the prime organizer of real-time PCR workshops globally. TATAA Biocenter conducts commissioned research and training within field of molecu-

lar diagnostics and gene expression analysis, along with developing real-time PCR expression panels. TATAA Biocenter has great experience and expertise in high resolution gene expression profiling, pathogen detection, and small sample/single cell analysis.



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