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Product Information

GloMelt™ Thermal Shift Protein Stability Kit

GloMelt™ Kit:

Component	Cat. No. 33021-T	Cat. No. 33021-1
GloMelt™ Dye, 200X	99843-20uL (200 x 20 uL reactions)	99843-200uL (2000 x 20 uL reactions)
Goat IgG Control, 10 mg/mL	99844-20uL	99844-20uL

GloMelt™ Kit (with ROX):

Component	Cat. No. 33022-T	Cat. No. 33022-1
GloMelt™ Dye, 200X	99843-20uL (200 x 20 uL reactions)	99843-200uL (2000 x 20 uL reactions)
Goat IgG Control, 10 mg/mL	99844-20uL	99844-20uL
ROX Reference Dye, 40 uM	99845-200uL	99845-1mL

Storage and Handling

Store GloMelt™ dye at -20°C, protected from light. GloMelt™ dye is stable for up to 2 years from date of receipt when stored as recommended. GloMelt™ dye is a potentially harmful chemical; exercise general laboratory safety precautions when handling, and dispose as hazardous chemical waste according to your local regulations. GloMelt™ dye is supplied at 200X in water with 20% DMSO.

Store ROX Reference Dye at -20°C, protected from light. ROX is stable for up to 2 years from date of receipt when stored as recommended. ROX is supplied at 40 uM in 10 mM Tris-HCL, 0.1 mM EDTA.

Store Goat IgG Control at -20°C. After thawing, it can be kept at 4°C for up to 2 years, or refrozen. Avoid multiple freeze-thaw cycles. Goat IgG Control protein is supplied at 10 mg/mL in 10 mM sodium phosphate, 0.15 M sodium chloride, pH 7.2, 0.05% sodium azide.

Spectral Properties

GloMelt™ Dye Abs/Em: 468/507 nm

Product Description

GloMelt™ dye undergoes fluorescence enhancement upon binding to hydrophobic regions of denatured proteins. Therefore the dye can be used to detect protein unfolding or measure thermal stability by performing a thermal shift assay, also called Protein Thermal Shift™, differential scanning fluorimetry, or Thermofluor assay.

The thermal shift assay is a rapid and inexpensive technique that quantifies change in protein denaturation temperature, and can therefore be used to screen conditions that affect protein thermal stability, such as protein mutations, ligand binding, and buffer formulations (like pH, salts, detergents, and other additives). These assays are rapid (typically about 30 minutes) and are performed on a quantitative PCR system. The thermal shift method is compatible with high-throughput screening and requires much less protein than methods such as circular dichroism and differential scanning calorimetry.

GloMelt™ dye has significant advantages over other environmentally sensitive dyes, such as SYPRO® Orange and PROTEOSTAT® TS dye. GloMelt™ dye generates a strong signal because it is optimized for detection in the SYBR® Green channel of qPCR instruments, and therefore low reaction volumes and low protein concentrations can be used. GloMelt™ dye is compatible with high concentrations of protein stabilizers (such as glycerol and sorbitol), and also protein destabilizers (such as DTT and imidazole). GloMelt™ dye performs very well in high detergent concentrations, unlike SYPRO® Orange. Another advantage is that ROX dye can be included with GloMelt™ dye during thermal shift assays, which improves results by increasing replicate consistency in qPCR instruments that require ROX passive reference dye.

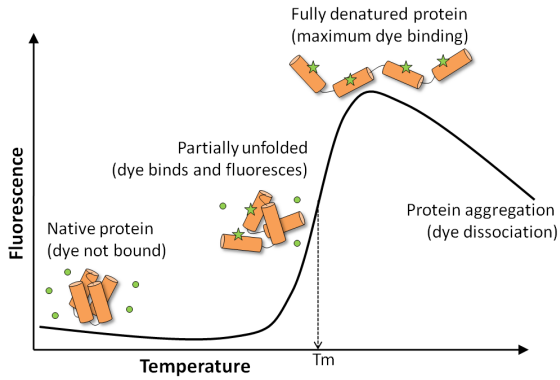

Figure 1. Environmentally sensitive fluorescent dyes can be used to monitor the temperature dependent unfolding of a protein. The protein's melting temperature (Tm) is a reporter of the protein's thermal stability.

Reaction Setup

1. Prepare 1X GloMelt™ working solution.

   Allow GloMelt™ dye to thaw and reach room temperature. Vortex and centrifuge vial briefly. Using your buffer of choice (such as PBS, TE, or water), freshly prepare sufficient 10X dye working stock for the number of samples to be assayed. For example; to prepare sufficient 10X dye for 50 reactions of 20 uL each; add 5 uL 200X dye to 95 uL PBS; and use 2 uL 10X dye per tube for a final 1X dye concentration.

   Note:
   We recommend using a final concentration of 1X dye in your reaction, but final concentrations of 0.5-2X may be tested to optimize your assay. We also recommend a reaction volume of 20 uL to conserve reagents, but volumes of up to 100 uL may be used.

2. Prepare the protein of interest in your buffer of choice.

   Dilute your protein to a final concentration of 0.5-2.5 ug/uL in your assay. Higher concentrations up to 5 ug/uL may also be used. For assays with IgG, we have found that a final concentration of 0.5-1 ug/uL works well.

   Note:
   For best results, the test protein should have an estimated purity of > 80% and should be free of precipitates. The GloMelt™ kit includes IgG control protein that can be used for initial assay setup. It is not necessary to run an IgG control sample with each experiment.

3. Set up reactions in qPCR tubes or a qPCR plate with a optical seal.

   Reactions contain dye, test protein, buffer, and may also contain test additive and ROX (as shown under General Reaction Setup). We recommend that no protein control reactions (NPCs) are prepared, containing all reaction components except test protein. At least 3 replicates of each reaction should be prepared, to test consistency and to obtain statistically significant results.

   Note:
   We recommend the reaction setup to be done on ice if the ambient temperature stability of your protein is unknown. If your protein is thermally stable at ambient temperatures, reaction setup can be performed at room temperature.

4. Run a melt curve profile. Setup will depend on your instrument.

   Spin tubes briefly and place in a real-time PCR thermocycler. See Protein Melt Run Profile table for a general setup. For proteins with high melting temperatures the initial hold and starting melt temperature can be increased. Acquire data continuously during the melt curve step.

   Note:
   Depending on your thermocycler, acquisition should be in the blue channel or green channel (with Ex/Em of ~470/510 nm), or the reporter should be set to SYBR® Green or FAM™. Set the passive reference to "None", or to "ROX" if ROX dye has been included in the assay. The ramp rate can affect the protein denaturation and should be optimized for your test protein. Ramp rates of between 0.01-0.05°C/s are commonly used.

Example of Simple Reaction Setup for IgG

Reaction component	Amount required per 20 uL reaction	Final concentration
IgG (10 ug/uL)	1 uL (10 ug)	0.5 ug/uL
GloMelt™ dye (10X)	2 uL	1X
Buffer of choice (1X)	17 uL	1X

General Reaction Setup

Reaction component	Amount required per 20 uL reaction	Final concentration
Test protein	x uL (10-100 ug)	0.5-5 ug/uL
GloMelt™ dye (10X)	1-4 uL	0.5-2X
Additive	Optional	See Table 1. Reagent Compatibility
ROX reference dye	Optional	See note [a] and Table 2
Buffer of choice	2 uL 10X buffer + H_2O to 20 uL final volume, or 1X buffer to 20 uL	

[a] During quantitative PCR, many qPCR systems require ROX dye to compensate for well-to-well optical variation. In thermal shift assays ROX is optional, however, including it can reduce standard deviation between replicates. Refer to Table 2 for the recommended ROX concentration for your qPCR instrument.

Table 1. Reagent Compatibility

Reagent	Compatible Concentration
CHAPS	1% [a]
DNA	0.1 ng/uL [b]
DTT	Up to 200 mM was tested
EDTA	Up to 400 mM was tested
Glycerol	Up to 50% was tested
Glycine	Up to 1 M was tested
Guanidine thiocyanate	Up to 25 mM tested
Imidazole	Up to 500 mM tested
PMSF	1 mM
Sodium Chloride	Up to 2 M was tested
Sorbitol	Up to 50% was tested
Sucrose	Up to 50% was tested
Trehalose	Up to 50% was tested
Triton™ X-100	0.5% [a]
TWEEN® 20	1% [a]
TWEEN® 80	1% [a]
Urea	Up to 1 M was tested

[a] Detergent may increase background fluorescence.
[b] DNA may increase background fluorescence.

Protein Melt Run Profile

Profile step	Temperature	Ramp rate	Holding Time
Initial hold	25°C	N/A	30 s
Melt/dissociation curve	25-99°C	1% or 0.05°C/s	N/A

Data Analysis

1. Analyze the fluorescence vs temperature data. In Applied Biosystems/ Thermo Fisher Scientific software, this is the "normalized reporter" melt curve plot.

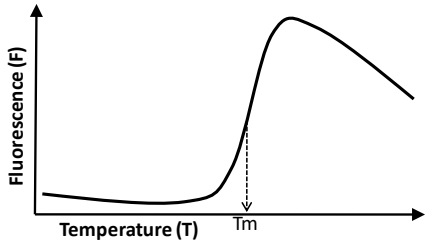

Figure 2. Fluorescence vs temperature.

2. Generate the protein melt curve plot using first derivative (slope) of the fluorescence curve.

   If available, use software designed for protein melt analysis, such as Protein Thermal Shift™ software (Thermo Fisher Scientific). Most real-time thermocyclers have software for DNA melt curve generation and analysis; this may also be used to analyze protein melt curves. These programs use the first derivative (slope) of the fluorescence curve (-dF/dT) to calculate the Tm of the DNA product.

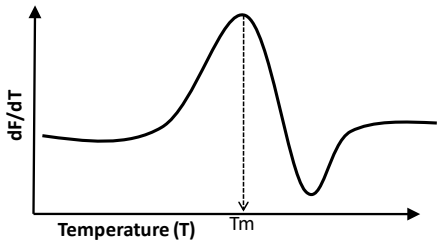

Figure 3. First derivative (slope) of the fluorescence curve (dF/dT) vs temperature.

   Note:
   The DNA melt curve software reports the negative of the first derivative because loss of fluorescence is detected, however, the protein melt curve measures gain in fluorescence. If using DNA melt curve software to analyze protein melting, the Tm will be at the lowest -dF/dT value (at the lowest point on the curve). Some software may have the ability to change the sign, and plot dF/dT (instead of -dF/dT); in this case the Tm is the temperature corresponding to the highest dF/dT value (peak of the curve, see Fig 3). If the software is not compatible with protein melt curve analysis, you may need to export the raw data and manipulate it using software such as Microsoft Excel®. Free online tools, such as DMAN (http://www.structuralchemistry.org/pcsb/dman.php) can also be used for data analysis.

Expected Results

GloMelt™ dye was evaluated extensively by thermal shift assay using IgG. Results will be dependent on test protein, and can vary by signal strength, background fluorescence levels, and melting temperature.

For the fluorescence vs temperature plot (or "normalized reporter" melt curve plot), the ideal curve will have low initial background fluorescence (at low temperature), followed by a peak confirming that your protein has unfolded.

Note:
If ROX was used as a reference dye, we recommend that you examine the the signal in the ROX or orange channel. The ROX fluorescence should be stable over the course of the assay, without obvious reactivity with the test protein or buffer additive.

Troubleshooting

Problem	Solutions
ROX signal is low or has large deviations	Issues with ROX signal may indicate 1) ROX was not added, 2) ROX is interacting with the protein or buffer additives. Set passive reference to "none" to permit data analysis without ROX normalization.
High fluorescence signal at low temperature	A high fluorescence signal at low temperature may indicate 1) your test protein has external hydrophobic regions in its native state, 2) your protein is already partially unfolded, 3) the GloMelt™ dye is interacting with components in the buffer. Confirm that your test protein does not contain protein stabilizers, such as BSA, that could lead to high background fluorescence signal. In some instances, initial background fluorescence may start high, but will decrease with increasing temperature, with a significant melt peak detectable at high temperature.
No sigmoidal transition to the unfolded protein state	A lack of sigmoidal transition may indicate the protein lacks a compact, globular folded structure; or the protein lacks sufficient hydrophobicity to generate a strong fluorescent signal. A curve without a melting peak may also indicate that your protein is very heat stable, with a Tm close to 99°C. Higher protein concentrations may be needed in the assay, or other methods of measuring protein thermal stability may be required, such as differential scanning calorimetry.

Table 2. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended ROX Concentration	Amount of ROX per 20 uL reaction
BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX-96 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCyler® Roche: LightCycler® 480, LightCycler® 2.0	No ROX	None Required
Thermo Fisher Scientific (ABI): 7500, 7500 Fast, ViiA 7™, QuantStudio™ Stratagene: MX4000P, MX3000P, MX3005P	Low ROX (~50 nM)	Dilute 40 uM ROX 1/100 with dH_2O or buffer, then add 2.5 uL diluted ROX (400 nM) per 20 uL thermal shift reaction.
Thermo Fisher Scientific (ABI): 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne®, StepOnePlus®	High ROX (~500 nM)	Dilute 40 uM ROX 1/10 with dH_2O or buffer, then add 2.5 uL diluted ROX (4 uM) per 20 uL thermal shift reaction.

Related Products

Catalog No.	Product
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
21003	One-Step Blue® Protein Gel Stain
21004	One-Step Lumitein™ Protein Gel Stain
21005	One-Step Lumitein™ UV Protein Gel Stain
22001	Ponceau S Solution
30071	AccuOrange™ Protein Quantitation Kit
22010	10X Fish Gelatin Blocking Agent
22012	Non-fat Dry Milk Powder
22011	Fish Gelatin Powder
22014	Bovine Serum Albumin, 30% Solution
22002	TWEEN® 20
41003	GelRed® Nucleic Acid Gel Stain
41005	GelGreen® Nucleic Acid Gel Stain
41008	PAGE GelRed® Nucleic Acid Gel Stain
80033	Thioflavin T "High Purity Grade"
80030	DCDAPH
80028	Congo Red "High Purity Grade"
22020	10X Phosphate-Buffered Saline (PBS)
41024-4L	Water, Ultrapure Molecular Biology Grade

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