SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Product Information

GloMelt™ Thermal Shift Protein Stability Kit

GloMelt™ (No ROX) Kit Contents

Component	33021-T 200 x 20 uL reactions	33021-1 2000 x 20 uL reactions
GloMelt™ Dye, 200X	99843-20UL 1 x 20 uL	99843-200UL 1 x 200 uL
Goat IgG Control, 10 mg/mL	99844-20L 1 x 20 uL	99844-20UL 1 x 20 uL

GloMelt™ (With ROX) Kit Contents

Component	33022-T 200 x 20 uL reactions	33022-1 2000 x 20 uL reactions
GloMelt™ Dye, 200X	99843-20UL 1 x 20 uL	99843-200UL 1 x 200 uL
Goat IgG Control, 10 mg/mL	99844-20UL 1 x 20 uL	99844-20UL 1 x 20 uL
ROX Reference Dye, 40 uM	99845-200UL 1 x 200 uL	99845-1ML 1 x 1 mL

Storage and Handling

Store at -20°C. Protect GloMelt™ Dye and ROX Reference Dye from light. Kit components are stable for at least 2 years from date of receipt when stored as recommended.

GloMelt™ Dye is supplied at 200X in water with 20% DMSO. GloMelt™ Dye is a potentially harmful chemical. Exercise universal laboratory safety precautions when handling the dye and dispose of hazardous chemical waste according to your local regulations.

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

ROX is supplied at 40 uM in 10 mM Tris-HCl, 0.1 mM EDTA.

Absorption/Emission: 468/507 nm (GloMelt™ Dye)

Product Description

GloMelt™ Dye undergoes fluorescence enhancement upon binding to hydrophobic regions of denatured proteins (Figure 1). 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. This rapid and inexpensive technique quantifies changes 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). The assay is rapid (typically about 30 minutes) and performed using quantitative PCR systems. 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), and performs very well in high detergent concentrations, unlike SYPRO® Orange (see Table 1 for reagent compatibility). Another advantage of GloMelt™ Dye is that it is detected in the SYBR®/FAM™ channel, allowing it to be combined with ROX passive reference dye for well-to-well normalization, which improves results by increasing replicate consistency in qPCR instruments that use ROX normalization.

Note that this assay is not compatible with transmembrane proteins due to exposed hydrophobic residues when isolated from their native membrane environment.

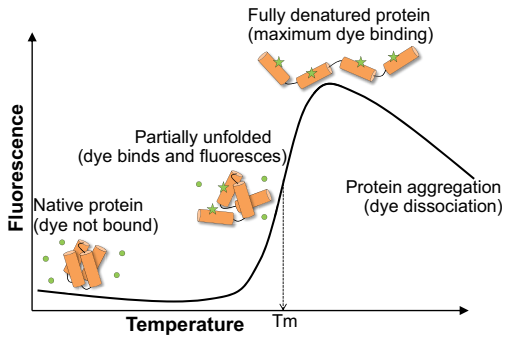

Figure 1. Environmentally sensitive fluorescent dyes can be used to monitor the temperature dependent unfolding of a protein. The protein's melting temperature (Tm) is a reporter of the protein's thermal stability.

Assay Considerations

- We recommend using a final concentration of 1X GloMelt™ 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.
- For best results, the test protein should have an estimated purity of > 80% and should be free of precipitates. Recommended protein concentration is 0.5-2.5 ug/uL, though higher concentrations of up to 5 ug/uL may be used. For assays with IgG, we have found that a final concentration of 0.5-1 ug/uL works well. You may use your buffer of choice (for example, PBS, TE, other buffer, or water) for preparing protein samples for assay.
- GloMelt™ has higher tolerance for detergents, reducing agents, and other protein additives compared to other thermal shift dyes. See Table 1 for reagent compatibility.
- At least 3 replicates of each reaction should be prepared, to test consistency and to obtain statistically significant results. We recommend that no protein control reactions (NPCs) are prepared, containing all reaction components except test protein.
- 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.
- Some qPCR thermocyclers require ROX passive reference dye to compensate for well-to-well optical variation. ROX is optional in thermal shift assays, but including it can reduce standard variation between replicates. Refer to Table 5 for the recommended ROX concentration for your 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.

Experimental Protocol

1. Instrument setup

1.1 GloMelt™ assay should be run on a real-time qPCR thermocycler. Acquisition for the reporter should be set to the SYBR® Green or FAM™ channel (Ex/Em of ~470/510 nm).

1.2 Set the passive reference to “None” if ROX is not used, or to “ROX” if ROX dye has been included in the assay.

1.3 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/second are commonly used. Set the instrument to acquire fluorescence data continuously during the melt curve step. See Table 2.

Table 2. Protein Melt Run Profile

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

2. Reaction setup

2.1 Allow kit components to thaw and reach room temperature. Vortex the vials to mix and centrifuge briefly to collect contents at the bottom of the vial.

2.2 Freshly prepare sufficient 10X GloMelt™ working solution for the number of samples to be assayed by diluting the 200X dye stock 1:200 in your buffer of choice. You will need 2 uL of 10X GloMelt™ working solution for each 20 uL reaction. For example, combine 5 uL of 200X GloMelt™ Dye with 95 uL of buffer (enough for 50 reactions). Volumes may be scaled as needed.

2.3 If using ROX, in a separate tube, prepare 10X ROX (0.5 uM or 5 uM, depending on your qPCR instrument) working solution (volumes may be scaled as needed):

a. For low ROX instruments, prepare 10X ROX (0.5 uM) by diluting the 40 uM ROX stock 1:80 in your buffer of choice. For example, combine 1.25 uL of 40 uM ROX with 98.75 uL of buffer for 0.5 uM dye (enough for 50 reactions).

b. For high ROX instruments, prepare 10X ROX (5 uM) by diluting the 40 uM ROX stock 1:8 in your buffer of choice. For example, combine 12.5 uL of 40 uM ROX with 87.5 uL of buffer for 5 uM dye (enough for 50 reactions).

2.4 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 (see Assay Considerations).

2.5 Set up reactions in qPCR tubes or a qPCR plate. Reactions contain dye, test protein, buffer, and may also contain test additive and ROX. See Table 3 for general reaction setup.

2.6 Optional: Set up reactions for the Goat IgG Control. See Table 4 for example control IgG reaction setup.

2.7 Mix reactions well by pipetting up and down or gentle vortexing. Make sure there are no bubbles in the reaction tubes, which can cause erroneous fluorescence readings.

2.8 Seal the tubes or plate, centrifuge briefly, and place in the thermocycler. Run the melt curve reaction.

Table 3. Example of General Reaction Setup

Component	Volume per 20 uL reaction	Final concentration
Test protein	x uL (10-100 ug)	0.5-5 ug/uL
Additive (optional)	Variable	See Table 1
10X GloMelt™ Dye[1]	1-4 uL	0.5-2X
10X ROX Reference Dye (optional)[2]	2 uL	50 nM (low ROX) or 500 nM (high ROX)
Buffer of choice	2 uL of 10X buffer + water to 20 uL; Or 1X buffer to 20 uL	

[1]See protocol step 2.2
[2]See protocol step 2.3

Table 4. Example of Control IgG Reaction Setup

Component	Volume per 20 uL reaction	Final concentration
Goat IgG Control (10 mg/mL)	1 uL	0.5 ug/uL
10X GloMelt™ Dye[1]	2 uL	1X
10X ROX Reference Dye (optional)[2]	2 uL	50 nM (low ROX) or 500 nM (high ROX)
PBS	17 uL (no ROX) or 15 uL (with ROX)	1X

[1]See protocol step 2.2
[2]See protocol step 2.3

3. Data analysis

3.1 Analyze the fluorescence vs temperature data. In Applied Biosystems/Thermo Fisher Scientific software, this is the "normalized reporter" melt curve plot (Figure 2).

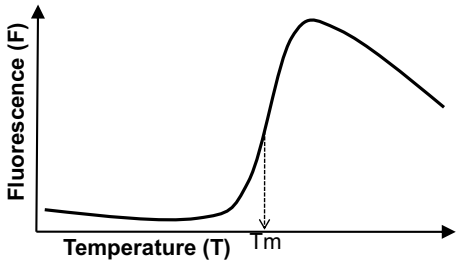

Figure 2. Fluorescence vs temperature.

3.2 Generate the protein melt curve plot using first derivative (slope) of the fluorescence curve (Figure 3).

Notes:

a. If available, use software designed for protein melt analysis, such as Protein Thermal Shift™ software.

b. 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 a DNA product. 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.

c. 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 Figure 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) also are available for data analysis.

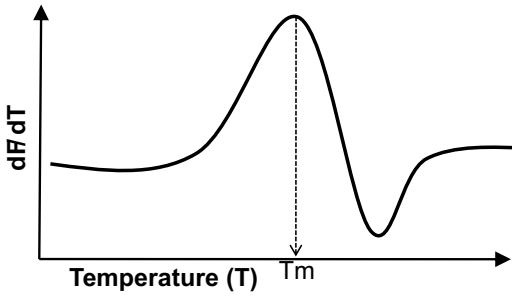

Figure 3. First derivative (slope) of the fluorescence curve (dF/dT) vs temperature.

Table 5. Recommended ROX Concentration for qPCR Instruments

qPCR 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: SmartCycler® Roche: LightCycler® 480, LightCycler® 2.0	None	None
Thermo Fisher Scientific (ABI): 7500, 7500 Fast, ViiA 7™, QuantStudio™ Stratagene: MX4000P, MX3000P, MX3005P	Low ROX (50 nM)	Dilute 40 uM ROX 1:80 in buffer, add 2 uL per 20 uL reaction
Thermo Fisher Scientific (ABI): 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOnePlus	High ROX (500 nM)	Dilute 40 uM ROX 1:8 in buffer, then add 2 uL per 20 uL reaction

Expected Results

- GloMelt™ dye was evaluated extensively in 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.
- If ROX was used as a reference dye, we recommend that you examine 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, or 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, or 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 1) the protein lacks a compact, globular folded structure, or 2) 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.

Related Products

Cat. No.	Product
33027, 33028	GloMelt™ 2.0 Thermal Shift Protein Stability Kit
30071	AccuOrange™ Protein Quantitation Kit
21003	One-Step Blue® Protein Gel Stain
21004	One-Step Lumitein™ Protein Gel Stain
21005	One-Step Lumitein™ UV Protein Gel Stain
33025, 33026	VersaBlot™ Total Protein Normalization Kits
22001	Ponceau S Solution
23013	TrueBlack® WB Blocking Buffer Kit
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
80033	Thioflavin T, High Purity Grade
80030	DCDAPH
80028	Congo Red "High Purity Grade"
22011	Fish Gelatin Powder
22014	Bovine Serum Albumin, 30% Solution
22002	Tween® 20
41024-4L	Water, Ultrapure Molecular Biology Grade

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and reactive dyes, protein and antibody labeling kits, fluorescent probes, background quenchers, and other accessories for protein analysis.