TAF1 bromodomains 1 and 2 TR-FRET Assay Kit

Item No. 600930

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	384 wells Quantity/Size	1,920 wells Quantity/Size	9,600 wells Quantity/Size	Storage
600931	TAF1 bromodomains 1 and 2 Europium Chelate	1 vial/420 wells	5 vials/ 420 wells	5 vials/ 2,100 wells	-80°C
600932	TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture	1 vial/420 wells	5 vials/ 420 wells	5 vials/ 2,100 wells	-80°C
600503	TR-FRET Assay Buffer (10X)	1 vial/2 ml	1 vial/10 ml	5 vials/10 ml	-20°C
600504	TR-FRET Assay Buffer Additive	1 vial/200 mg	1 vial/1 g	5 vials/1 g	-20°C
600507	H4 Positive Control	1 vial/2.5 nmol	5 vials/2.5 nmol	5 vials/12.5 nmol	-80°C
400093	384-Well Solid Plate (low volume; black)	1 plate	5 plates	25 plates	Room temperature
400023	Foil Plate Covers	1 cover	5 covers	25 covers	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's TAF1 bromodomains 1 and 2 TR-FRET Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of time-resolved FRET with an excitation at 340 nm and emission at 620 and 670 nm
2. Adjustable pipettes and a multichannel pipettor
3. DMSO

INTRODUCTION

Background

The acetylation of histone lysine residues plays a crucial role in the epigenetic regulation of gene transcription. Acetylated lysine residues are recognized by a small protein domain known as a bromodomain.[1] These domains recruit regulatory complexes to acetylated nucleosomes, thereby controlling chromatin structure and gene expression. TAF1 is the largest subunit of transcription factor IID and binds to the core promoter sequences at the transcription start site.[2] In addition to its two bromodomains, this large protein (250 kDa) possesses a kinase domain, a histone acetyltransferase domain and E1/E2 ubiquitin-activation/conjugation domains.[3] Through its interactions with the androgen receptor, TAF1 has been implicated in castration-resistant prostate cancer.[3] TAF1 also has been shown to regulate expression of the pro-apoptotic protein $p27^{kip1}$, potentially linking TAF1 expression to the cellular response to genotoxic stress.[2]

About This Assay

Cayman's TAF1 bromodomains 1 and 2 TR-FRET Assay Kit is a homogeneous, Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) assay method amenable to rapid characterization of inhibitors of bromodomain/acetylated peptide interaction in a high throughput format. The 'donor' fluorophore in this assay consists of TAF1 bromodomains 1 and 2 (human amino acids 1,373-1,635) directly labeled with a europium (Eu^{3+}) chelate. A biotinylated peptide containing target acetylated lysine serves as the ligand for the TAF1 bromodomains 1 and 2. Allophycocyanin (APC)-labeled avidin binds with high affinity to the peptide substrate *via* the biotin moiety and serves as the 'acceptor' fluorophore in the assay. Inhibition of the bromodomain/peptide interaction displaces BRD-Eu^{3+} from the APC/avidin resulting in a loss of TR-FRET signal. The TAF1 bromodomains 1 and 2 TR-FRET Assay Kit is robust (Z' >0.6), and is suitable for high-throughput screening in the provided 384-well plate or can be scaled to higher density plate formats (*e.g.*, 1,536-well plate) if desired. The assay is stable at room temperature for at least four hours and in the presence of less than 2% DMSO.

Introduction to TR-FRET

TR-FRET is based upon the principles of FRET, but possesses a number of advantages that make it a superior technology for high-throughput screening. When an optically active molecule absorbs a photon it has several options by which it may release that energy: it may release a photon of a longer wavelength (less energy) than the photon it absorbed, it may dissipate the energy as heat, or it can transfer the energy non-radiometrically to a suitable acceptor fluorophore. The latter effect is known as FRET and is a commonly used phenomenon in biological assays. In these assays, a donor flurophore is coupled to one binding partner and an acceptor fluorophore is coupled to the other binding partner. The binding partners are mixed in an assay well and allowed to associate. The donor fluorophore is then excited with a wavelength of light that does not excite the acceptor fluorophore and if the molecules are within approximately 100 Å of each other, the donor fluorophore can non-radiometrically transfer the energy to the acceptor fluorophore, which will then release that photon as light with at a wavelength characteristic of the acceptor fluorophore (see Figure 1 on page 8). For each assay point, the fluorescence intensity of the donor fluorophore and the acceptor fluorophore are measured and the data are generally presented as the ratio of acceptor fluorophore intensity/donor fluorophore intensity. This methodology is particularly sensitive because the FRET efficiency decays as the 6^{th} power of the distance between the two fluorophores. Therefore, unassociated binding partners are unlikely to lie within the distance required for efficient FRET.

TR-FRET is an extension of FRET that utilizes a donor fluorophore with a long fluorescent half-life. These fluorophores are based upon lanthanide (most often Eu^{3+} or Tb^{3+}) chelates that have characteristically large stokes shifts and fluorescent half-lives on the order of milliseconds. The long fluorescent lifetime allows the TR-FRET signal to be sustained for dramatically longer periods of time than standard fluorescence. This is particularly advantageous because it affords the ability to measure the TR-FRET signal after background fluorescence in the assay (*e.g.*, buffer/reagent autofluorescence) has dissipated (see Figure 2 on page 9). The increased signal:noise ratio and the diminished effects of screening compound fluorescence makes TR-FRET assays particularly useful for high-throughput screening applications.

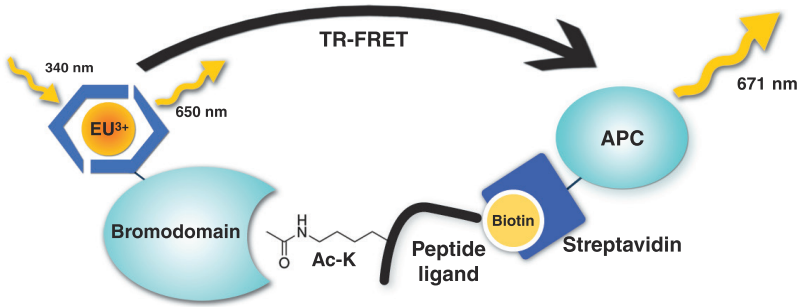

Figure 1. Assay schematic for the bromodomain TR-FRET Assay Kit. Upon excitation, the europium chelate can release a photon or transfer its energy to an APC molecule, provided the APC is in close proximity to the europium fluorophore.

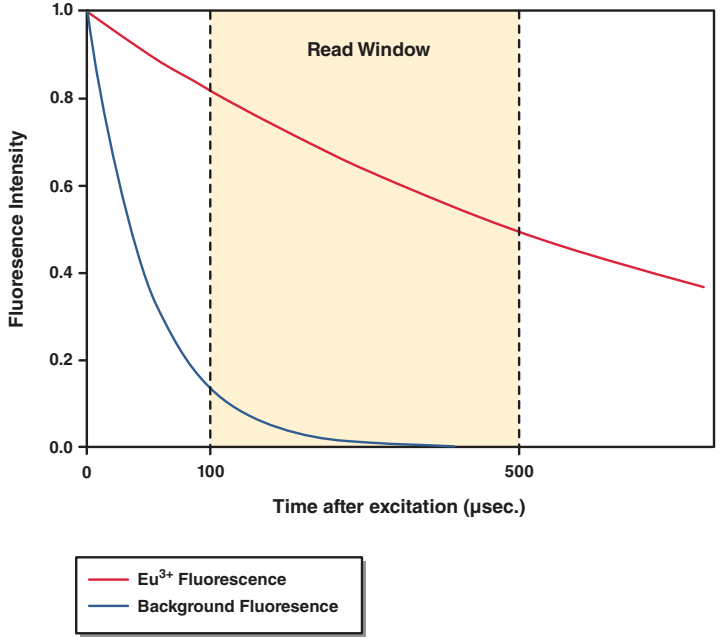

Figure 2. The extended fluorescence lifetimes of Eu^{3+}-based fluorophores allows the samples to be analyzed after background fluorescence has decayed, improving signal to noise and reducing spectral artifacts.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). UltraPure water may be purchased from Cayman Chemical (Item No. 400000).

Buffer Preparation

2 ml vial TR-FRET Assay Buffer (10X) (384-well kit; Item No. 600503): Add 18 ml of UltraPure water to the vial. Add 200 mg of TR-FRET Assay Buffer Additive (Item No. 600504) and mix to dissolve. For best results, filter completed 1X Assay Buffer with a 0.22 µm filter before use. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.* Store the diluted buffer at 4°C; it will be stable for approximately one month.

OR

10 ml vial TR-FRET Assay Buffer (10X) (1,920- or 9,600-well kit; Item No. 600503): For five 384-well plates, dilute 10 ml TR-FRET Assay Buffer to a total volume of 100 ml with UltraPure water. Add 1 g of TR-FRET Assay Buffer Additive (Item No. 600504) and mix to dissolve. For best results, filter completed 1X Assay Buffer with a 0.22 µm filter before use. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.* Store the diluted buffer at 4°C; it will be stable for approximately one month.

Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in 1X TR-FRET Assay Buffer at a concentration 4X the desired final assay concentration (*e.g.*, for 1 µM final assay concentration, a 4 µM dilution should be made). This solution may contain up to 8% organic solvents such as DMSO, DMF, or short chain alcohols. The final concentration of organic solvents in the assay will then be <2%. Avoid using high concentrations of metal chelating agents or phosphate buffers.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

TAF1 bromodomains 1 and 2 Europium Chelate (Item No. 600931)

420-well vial TAF1 bromodomains 1 and 2 Europium Chelate (384- or 1,920-well kit):

On ice, thaw one tube of TAF1 bromodomains 1 and 2 Europium Chelate (420 wells) per 384-well plate and briefly centrifuge before opening. Dilute contents to a final volume of 4.2 ml in 1X TR-FRET Assay Buffer. Content volume is indicated on the label. Mix gently (do not vortex) and keep on ice. Diluted protein should be used within the same day.

OR

2,100-well vial TAF1 bromodomains 1 and 2 Europium Chelate (9,600-well kit):

On ice, thaw one tube of TAF1 bromodomains 1 and 2 Europium Chelate (2,100 wells) per five 384-well plates and briefly centrifuge before opening. Dilute contents to a final volume of 21 ml in 1X TR-FRET Assay Buffer. Content volume is indicated on the label. Mix gently (do not vortex) and keep on ice. Diluted protein should be used within the same day.

TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture (Item No. 600932)

420-well vial TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture (384- or 1,920-well kit):

For each 384-well plate, add 2.1 ml of 1X TR-FRET Assay Buffer to one vial of the TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture (420 wells) and gently vortex. Keep the solution in the dark to prevent photobleaching. Long-term storage of the diluted mixture is not recommended.

2,100-well vial TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture (9,600-well kit):

For five 384-well plates, add 3 ml of 1X TR-FRET Assay Buffer to one vial of the TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture (2,100 wells) and gently vortex. Transfer contents to a new tube and adjust the mixture to a final volume of 10.5 ml in 1X Assay Buffer. Keep the solution in the dark to prevent photobleaching. Long-term storage of the diluted mixture is not recommended.

H4 Positive Control (Item No. 600507)

2.5 nmol vial H4 Positive Control (384- or 1,920-well kit):

For each 384 well plate, add 200 µl of 1X TR-FRET Assay Buffer to one tube containing the H4 Positive Control (2.5 nmol) and vortex gently. Unused solutions may be stored at -20°C for approximately two weeks.

OR

12.5 nmol vial H4 Positive Control (9,600-well kit):

For five 384 well plates, add 1 ml of 1X TR-FRET Assay Buffer to one tube containing the H4 Positive Control (12.5 nmol) and vortex gently. Unused solutions may be stored at -20°C for approximately two weeks.

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Do not expose the pipette tip to the reagent(s) already in the well.
- Avoid introducing bubbles into the wells.

Follow the steps below to accurately measure the TR-FRET ratio in the assay. Allow all reagents except for TAF1 bromodomains 1 and 2 Europium Chelate to equilibrate to room temperature prior to performing the assay. Keep the TAF1 bromodomains 1 and 2 Europium Chelate on ice until just prior to use. NOTE: Volumes indicated below are for a 384-well plate format with a 20 µl final assay volume. The customer may scale as needed for higher or lower density plate formats.

1. Inhibitor Samples

Dilute inhibitor samples in 1X TR-FRET Assay Buffer to a concentration that is 4X the desired final concentration (*e.g.*, if 1 µM is desired, prepare a 4 µM solution). This solution may contain up to 8% of an organic solvent (*e.g.*, DMSO). Add 5 µl of this solution to the desired wells. For best results, perform the assay in duplicate.

It is recommended that inhibitor compounds be tested in a concentration-response format with at least eight independent concentrations that span approximately a 1,000-fold range around the expected IC_{50} value of the inhibitor.

2. Positive and Negative Control Samples

For positive (inhibitor control) control wells, add 5 µl of the H4 Positive Control to the desired wells. This will provide a final assay concentration of 3 µM H4 Positive Control.

For negative (no inhibition) control wells, add 5 µl of 1X TR-FRET Assay Buffer to the desired wells. If inhibitor samples from step 1 contain organic solvent, add an equivalent amount of the solvent into the assay in this step.

3. TAF1 bromodomains 1 and 2 Europium Chelate

Add 10 µl of the diluted TAF1 bromodomains 1 and 2 Europium Chelate to every well of the 384-well plate.

4. Pre-incubation (optional)

If desired, incubate the control and sample wells for 15 minutes at room temperature to allow pre-equilibration of the inhibitor and control compounds with the TAF1 bromodomains 1 and 2 Europium Chelate. *Protect from light.*

5. TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture

Add 5 µl of the reconstituted TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture to every well.

6. Incubation of the Plate

Seal the plate with an adhesive aluminum seal and incubate at room temperature for one hour. For automation purposes, the plate does not have to be sealed, but it should remain in the dark to prevent photobleaching.

7. Reading the Plate

Read the plate(s) in time-resolved format by exciting the sample at 340 nm and reading emissions at 620 and 670 nm, using a 100 µs delay and a 500 µs read window. To ensure optimal assay sensitivity, it is strongly recommended that a filter-based instrument be used to perform TR-FRET measurements. The plate reader used at Cayman Chemical employs a 340/40 nm excitation filter, 620/15 nm, and 671/20 nm emission filters. Samples will be stable for analysis for at least five hours if stored at room temperature and protected from light. Data analysis is performed using the TR-FRET ratio (670 nm emission/620 nm emission).

Well Type	H4 Positive Control (µl)	1X Assay Buffer (µl)	Test Sample (µl)	TAF1 bromodomains 1 and 2 Europium Chelate (µl)	TAF1 bromodomains 1 and 2 Ligand/APC Acceptor Mixture (µl)
Positive Control	5	-	-	10	5
Negative Control	-	5*	-	10	5
Experimental Samples	-	-	5	10	5

Table 1. Pipetting summary

*If an organic solvent is used at concentrations >2% in the test samples, include it in the negative control wells at the same concentration as the sample wells to control for solvent effects.

Effects of Solvents

Samples may be prepared in organic solvents such as DMSO, DMF, or short chain alcohols (*e.g.*, MeOH, EtOH), as long as the final concentration of organic solvents in the assay is <2%. High concentrations of metal chelating agents or phosphate buffers may interfere with the fluorescence of the donor fluorophore and should be avoided. If conditions require different solvents or higher concentrations, additional assays may be required to assess solvent interference.

ANALYSIS

Calculations

A plot of the TR-FRET ratio (670 nm emission/620 nm emission) *versus* inhibitor concentration on semi-log axes results in a sigmoidal dose-response curve typical of competitive binding assays. This data can be fit to a 4-parameter logistic equation as shown in Figure 3 to calculate IC_{50} values.

Performance Characteristics

Z' Factor:

Z' factor is a term used to describe the robustness of an assay,[4] which is calculated using the equation below.

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{\left| \mu_{c+} - \mu_{c-} \right|}$$

Where c+: Positive control

c-: Negative control

The theoretical upper limit for the Z′ factor is 1.0. A robust assay has a Z′ factor >0.5. The Z′ factor for Cayman's TAF1 bromodomains 1 and 2 TR-FRET Assay Kit was determined to be 0.77.

Sample Data

The data shown here is an example of the data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

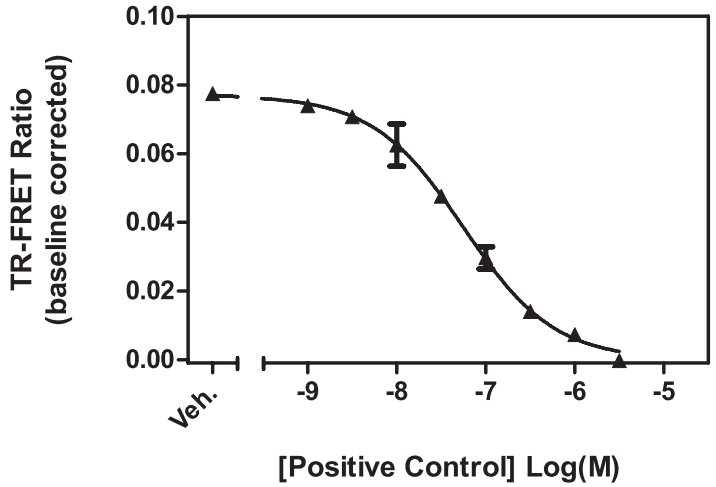

Figure 3. Typical inhibition curves for the displacement of the acetylated peptide from TAF1 bromodomains 1 and 2 by the H4 Positive Control. "Veh." represents compound vehicle control.

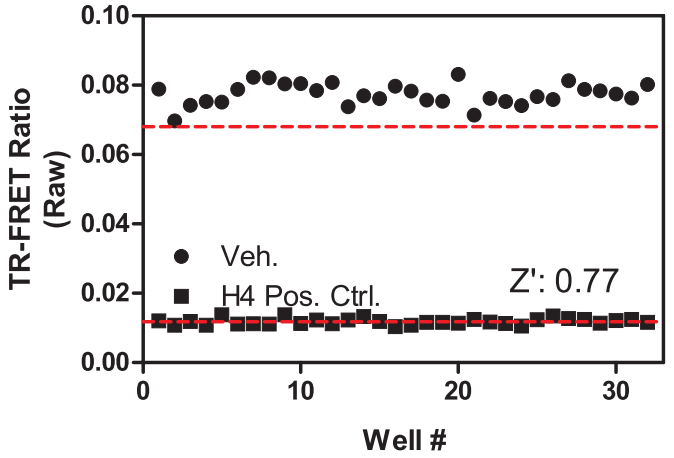

Figure 4. Typical Z' data for the TAF1 bromodomains 1 and 2 TR-FRET Assay Kit. Data are shown from 32 replicate wells of both positive and negative controls prepared as described in the kit booklet. The calculated Z' factor from this experiment was 0.77. The red lines correspond to three standard deviations from the mean for each control value.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Bubble in the well B. Poor pipetting/technique	A. Centrifuge the plate briefly
Low fluorescence signal	A. Incompatible sample matrix B. TAF1 bromodomain protein handled improperly C. Monochromater-based instrument used for data acqusition	A. Test sample matrix for interference before running samples in the assay B. Keep the protein frozen at -80°C until ready to use; thaw protein and keep on ice until adding to assay C. Analzye the assay using a filter-based plate reader

References

1. Mujtaba, S., Zeng, L., and Zhou, M.-M. Structure and acetyl-lysine recognition of the bromodomain. *Oncogene* **26**, 5521-5527 (2011).
2. Kimura, J., Nguyen, S.T., Liu, H., *et al.* A functional genome-wide RNAi screen identifies TAF1 as a regulator for apoptosis in response to genotoxic stress. *Nucleic Acids Res.* **36(16)**, 5250-5259 (2008).
3. Tavassoli, P., Wafa, L.A., Cheng, H., *et al.* TAF1 differentially enhances androgen receptor transcriptional activity *via* its N-terminal kinase and ubiquitin-activating and -conjugating domains. *Mol. Endocrinol.* **24(4)**, 696-708 (2010).
4. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).

Related Products

BAZ2B bromodomain TR-FRET Assay Kit - Item No. 600710
BRD2 bromodomain 1 TR-FRET Assay Kit - Item No. 600500
BRD2 bromodomains 1 and 2 TR-FRET Assay Kit - Item No. 600810
BRD2 bromodomain 2 TR-FRET Assay Kit - Item No. 600510
BRD3 bromodomain 1 TR-FRET Assay Kit - Item No. 600630
BRD3 bromodomains 1 and 2 TR-FRET Assay Kit - Item No. 600820
BRD3 bromodomain 2 TR-FRET Assay Kit - Item No. 600640
BRD4 bromodomain 1 TR-FRET Assay Kit - Item No. 600520
BRD4 bromodomains 1 and 2 TR-FRET Assay Kit - Item No. 600830
BRD4 bromodomain 2 TR-FRET Assay Kit - Item No. 600530
BRDT bromodomain 1 TR-FRET Assay Kit - Item No. 600650
BRG1 bromodomain TR-FRET Assay Kit - Item No. 600720
BRM bromodomain TR-FRET Assay Kit - Item No. 600730
CBP bromodomain TR-FRET Assay Kit - Item No. 600850
(+)-JQ1 - Item No. 11187
(-)-JQ1 - Item No. 11232
TAF1 bromodomain 1 TR-FRET Assay Kit - Item No. 600870
TAF1 bromodomains 1 and 2 (human recombinant) - Item No. 14494
For a complete list of related products please visit: www.caymanchem.com/catalog/600930

Warranty and Limitation of Remedy

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Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

NOTES