FTO (intracellular; human) EIA Kit

Item No. 579010

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Materials Supplied

Vial Number	Item	96 wells Quantity/Size	Storage
1	Anti-FTO (human) Precoated 96-Well Strip Plate	1 plate	4°C
2	Wash Buffer Concentrate (10X)	1 vial/50 ml	4°C
3	Dilution Buffer (5X)	1 vial/50 ml	4°C
4	Detection Antibody	1 vial/12 ml	4°C
5	Anti-Rabbit IgG/HRP Conjugate (100X)	1 vial/150 µl	4°C
6	FTO (human) EIA Standard	1 vial	4°C
7	Substrate Solution	1 vial/12 ml	4°C
8	Stop Solution	1 vial/12 ml	4°C
9	Quality Control Sample	1 vial	4°C
10	Lysis Buffer Concentrate (10X)	1 vial/12 ml	4°C
11	Plate Cover	3 covers	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. 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 FTO (intracellular; human) EIA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

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 if stored as directed and used before the expiration date indicated on the outside of the box. Reagents must be stored at 4°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted Wash Solution (see page 9) may be stored at room temperature for up to one month.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of pure water; glass distilled water or deionized water is acceptable
4. Phosphate buffered saline
5. Materials used for **Sample Preparation** (see page 10).

INTRODUCTION

Background

The FTO gene was first described as one of several genes deleted during insertional mutagenesis in the Fused toes (Ft) mouse mutation.[1] Now known as the fat mass and obesity-associated gene, it is abundantly expressed in most organs.[1,2] The gene product is an AlkB-like DNA/RNA demethylase with a strong preference for single-stranded DNA and RNA.[3-5] Single nucleotide polymorphisms (SNPs) in the first intron of the FTO gene contribute to childhood obesity and severe adult obesity.[2,6] Knockout or loss-of-function of FTO produces an autosomal-recessive lethal syndrome with multiple malformations and severe growth retardation.[7,8] FTO is the first locus unequivocally associated with adiposity, with activity within the central nervous system apparently regulating feeding behavior and energy expenditure.[9,10]

About This Assay

Cayman's FTO (intracellular; human) EIA Kit is an immunometric assay which can be used to measure FTO in human cell lysates. The assay exhibits a detection limit of 50 pg/ml and an assay range of 0-10 ng/ml.

Principle of the Assay

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for FTO (mouse anti-human FTO). This antibody will bind any FTO introduced into the well. Standards and samples are incubated on the antibody-coated plate, and the plate is then washed before addition of a purified polyclonal anti-FTO antibody to detect the captured FTO. An anti-rabbit IgG/HRP-conjugate is used to recognize the 'sandwiches'. The concentration of the analyte is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound anti-rabbit IgG/HRP conjugate, which in turn is proportional to the concentration of the FTO.

$$\textbf{Absorbance} \propto [\textbf{anti-rabbit IgG/HRP}] \propto [\textbf{FTO}]$$

A schematic of this process is shown in Figure 1, on page 8.

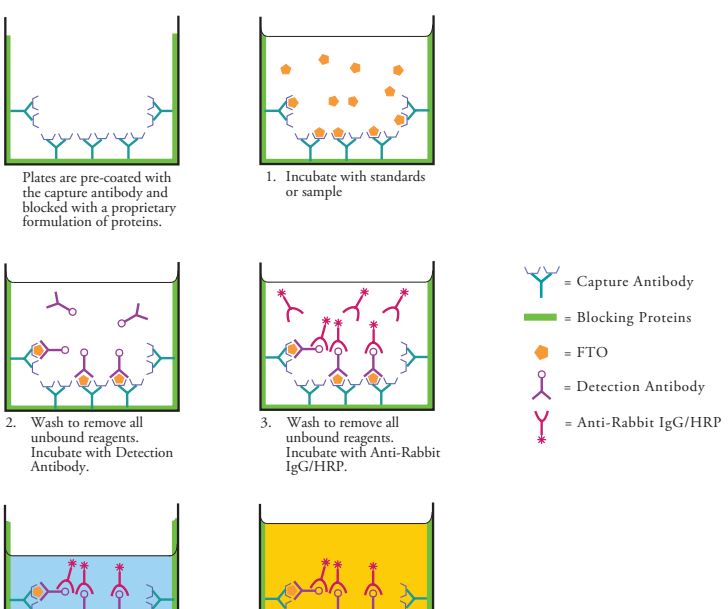

Figure 1. Schematic of the immunometric EIA

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months

1. Dilution Buffer Preparation

To prepare a 1X Dilution Buffer Solution, dilute the Dilution Buffer (5X) (vial #3) 1:5 with deionized water (*i.e.*, mix 1 part Dilution Buffer (5X) with 4 parts deionized water).

2. Wash Buffer Preparation

To prepare a 1X Wash Buffer Solution, dilute the Wash Buffer Concentrate (10X) (vial #2) 1:10 with deionized water (*i.e.*, mix 1 part Wash Buffer Concentrate (10X) with 9 parts deionized water).

3. Detection Antibody Preparation

Prior to use, warm the Detection Antibody (vial #4) to room temperature. The solution is ready to use as supplied.

4. Anti-Rabbit IgG/HRP Conjugate Preparation

Dilute the Anti-Rabbit IgG/HRP Conjugate (100X) (vial #5) 1:100 with 1X Dilution Buffer (*i.e.*, mix 1 part Anti-Rabbit IgG/HRP Conjugate (100X) with 99 parts 1X Dilution Buffer). Use the 1X Anti-Rabbit IgG/HRP Conjugate within one hour of preparation.

5. Substrate Solution Preparation

Prior to use, warm the Substrate Solution (vial #7) to room temperature. The solution is ready to use as supplied.

6. Lysis Buffer Preparation

To prepare a 1X Lysis Buffer Solution, dilute the Lysis Buffer Concentrate (10X) (vial #10) 1:10 with deionized water (*i.e.*, mix 1 part Lysis Buffer Concentrate (10X) with 9 parts deionized water). Add phenyl methylsulfonyl fluoride (PMSF) to a concentration of 1 mM immediately before use.

Sample Preparation

Cell Lysates

Grow cells to 90% confluency. Scrap cells off the plate and transfer to an appropriate microcentrifuge tube. Keep on ice and centrifuge at 1,200 rpm for five minutes at 4°C. Remove the supernatant and rinse cells once with ice-cold PBS. Remove the PBS and add 200 µl ice-cold 1X Lysis Buffer supplemented with 1 mM PMSF to ten million cells and incubate on ice for 30 minutes. Centrifuge at 12,000 rpm for five minutes at 4°C and transfer the supernatant (the cell lysate) to a new tube. Use freshly prepared cell lysate samples for analysis in the EIA.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

FTO (human) EIA Standard

When opening the lyophilized standard, remove the cap gently as the powder may be loose within the vial. Reconstitute the lyophilized purified FTO (human) EIA Standard (vial #6) with 1 ml of deionized water. Mix gently. The concentration of this solution (the bulk standard) is 20 ng/ml. We recommend that unused FTO EIA Standard be aliquotted and stored at -20°C.

To prepare the standard for use in EIA: Obtain eight clean microcentrifuge tubes and label them #1 through #8. Aliquot 300 µl of Dilution Buffer to tubes #1-8. Transfer 300 µl of freshly prepared bulk standard (20 ng/ml) to tube #8. Mix gently. The concentration of this standard is 10 ng/ml. Dilute the standard by removing 300 µl from tube #8 and placing into tube #7. Mix gently. Next, remove 300 µl from tube #7 and place into tube #6; mix gently. Repeat this process for tubes #5 to #2. Do not add any FTO to tube #1. This tube is the zero-point vial, the lowest point on the standard curve and will serve as the blank.

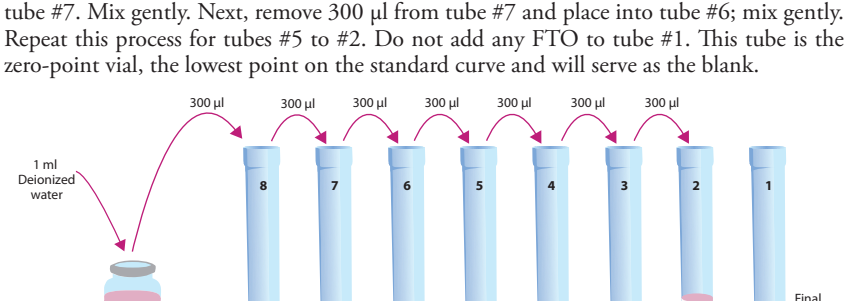

Figure 2. Preparation of the FTO (human) standards

Quality Control Sample

Reconstitute the Quality Control Sample (vial #9) in 1 ml of deionized water. Mix well. For actual concentration of the Quality Control Samples, see the enclosed Certificate of Analysis.

Sample Preparation

1. Dilute samples between 1:10 and 1:1,000 with 1X Dilution Buffer and mix well. If samples fall the outside range of assay, a lower or higher dilution may be required.
2. Use 100 µl of the final diluted sample in the EIA.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, place the unused strips back in the plate packet and store according to the plate insert at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 3, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 16, for more details). We suggest you record the contents of each well on the template sheet provided (see page 27).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	9	9	17	17	25	25	33	33
B	S2	S2	2	2	10	10	18	18	26	26	34	34
C	S3	S3	3	3	11	11	19	19	27	27	35	35
D	S4	S4	4	4	12	12	20	20	28	28	36	36
E	S5	S5	5	5	13	13	21	21	29	29	37	37
F	S6	S6	6	6	14	14	22	22	30	30	38	38
G	S7	S7	7	7	15	15	23	23	31	31	39	39
H	S8	S8	8	8	16	16	24	24	32	32	40	40

S1-S8 - Standards 1-8
1-40 - Samples

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of Standards and Samples and First Incubation

1. Add 100 µl of the standards, diluted sample, or QC sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with plate cover and incubate one hour at 37°C.

Addition of Detection Antibody and Second Incubation

1. Empty the wells and rinse three times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of the Detection Antibody to each well of the plate.
3. Cover the plate with plate cover and incubate for one hour at 37°C.

Addition of Anti-Rabbit IgG/HRP Conjugate and Third Incubation

1. Empty the wells and rinse three times with Wash Buffer.
2. Add 100 µl of Anti-Rabbit IgG/HRP Conjugate to each well of the plate.
3. Cover the plate with plate cover and incubate for one hour at 37°C.

Development of the Plate

1. Empty the wells and rinse five times with Wash Buffer.
2. Add 100 µl of Substrate Solution to each well of the plate.
3. Cover the plate with plate cover and incubate for 10 minutes at room temperature in the dark.
4. DO NOT WASH THE PLATE. Add 100 µl of Stop Solution (vial #8) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. We recommend using a second order polynomial (quadratic) fit since the absorbance at the standard concentrations is slightly non-linear. *NOTE: Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

1. Average the absorbance values for wells of the zero-point standard.
2. Subtract the average absorbance reading of the zero point standard from the absorbance of all wells of the plate.

Plot the Standard Curve

Plot absorbance *versus* concentration for standards (S1-S8) using linear x- and y-axis and fit the data with a quadratic equation. (Option: Plot concentration of the standard on the y-axis *versus* the absorbance on the x-axis.)

Determine the Sample Concentration

1. Use a calculator or spreadsheet to determine the quadratic regression line.
2. Use the equation of the curve generated by the regression fit to calculate the value of your samples. Be sure to correct for any dilution of the sample prior to addition to the well of the plate.

Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

FTO (ng/ml)	Raw Data	Blank Subtracted
0	0.165	0.0
0.156	0.208	0.043
0.313	0.241	0.076
0.625	0.343	0.178
1.25	0.55	0.385
2.5	0.896	0.731
5	1.482	1.317
10	2.223	2.058

Table 1. Typical results

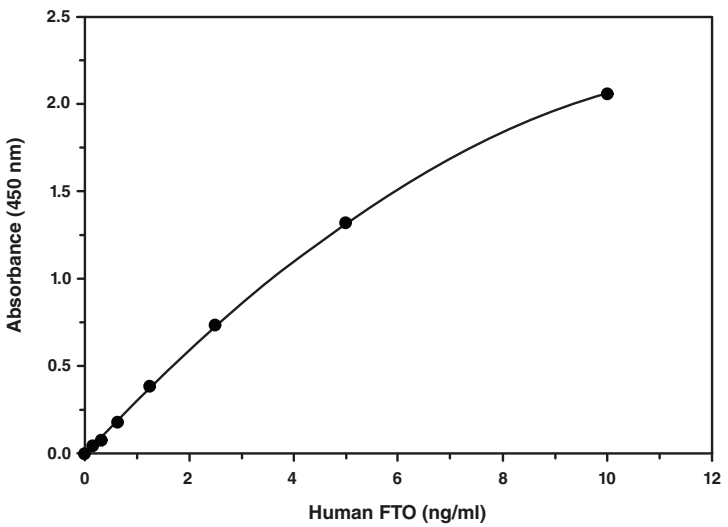

Figure 4. Typical standard curve
The minimum detectable concentration is 50 pg/ml.

Precision:

a. Intra-Assay Precision

Four samples were tested six times to assess the intra-assay precision.

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	%CV
1	32.0	1.8	5.6
2	262	6.1	2.3
3	96.3	1.6	1.6
4	131	2.8	2.2

Table 2. Intra-assay variation

b. Inter-Assay Precision

Three samples were tested four times to assess the inter-assay precision.

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	%CV
1	226	20.9	9.2
2	92.1	8.1	8.8
3	99.8	5.0	5.0

Table 3. Inter-assay variation

Specificity:

Analyte (recombinant proteins)	Max Concentration (ng/ml)	Cross Reactivity (%)
Human FTO	5	100
Mouse FTO	50	<5
Human Adiponectin	50	N.R.
Human ANGPTL3	50	N.R.
Human ANGPTL4	50	N.R.
Human Clusterin	50	N.R.
Human FGF21	50	N.R.
Human GPX3	50	N.R.
Human IDO	50	N.R.
Human IL-33	50	N.R.
Human Progranulin	50	N.R.
Human RBP4	50	N.R.
Human Resistin	50	N.R.
Human SIRT1	50	N.R.
Human Vaspin	50	N.R.
Human Nampt/Visfatin	50	N.R.
Mouse ANGPTL3	50	N.R.
Mouse Leptin	50	N.R.
Mouse Progranulin	50	N.R.
Rat Nampt/Visfatin	50	N.R.

Table 4. Specificity of the FTO (human) EIA Kit
N.R. = No Cross Reactivity

Recovery:

The recovery of FTO spiked to three different levels in two different samples throughout the range of assay was evaluated.

Sample	Average Recovery (%)	Range (%)
1	95.4	95-105
2	102	95-105
3	104	95-105

Table 5. Recovery of FTO (human)

Linearity - Effect of sample dilution:

To assess the linearity of the assay, three lysate samples were diluted as indicated below prior to sample preparation as described in the protocol.

Sample	Sample Dilution	Expected (ng/ml)	Observed (ng/ml)	% of Expected
Molt 4 cells	1:50	468	468	100
	1:100	234	211	90
	1:200	117	92.5	79
A549 cells	1:50	73.7	73.7	100
	1:100	36.8	30.2	82
	1:200	18.4	15	81.5
HepG2 cells	1:50	102	102	100
	1:100	51	41.2	80.8
	1:200	25.5	18.3	71.8

Table 6. Effect of lysate dilution (% of expected = observed/expected x 100%)

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal	A. Omission of key reagent B. Washes too stringent C. Incubation times inadequate D. Plate reader settings not optimal E. Incorrect assay temperature	A. Check that all reagents have been added in the correct order B. Use an automated plate washer if possible C. Use recommended incubation times D. Verify the wavelength and/or filter settings in the plate reader E. Use recommended incubation temperature; bring substrates to room temperature before use
High background	A. Concentration of Anti-Rabbit IgG/HRP too high B. Inadequate washing	A. Use recommended dilution B. Ensure all wells are filled with Wash Buffer and are aspirated completely
Poor standard curve	A. Wells not completely aspirated B. Reagents poorly mixed C. Dilution error D. Technique problem	A. Completely aspirate wells between steps B. Be sure reagents are thoroughly mixed C. Check pipetting technique and double-check calculations D. Proper mixing of reagents and wash steps are critical

References

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Related Products

Adipogenesis Assay Kit - Item No. 10006908
Adipolysis Assay Kit - Item No. 10009381
Adiponectin (human) EIA Kit - Item No. 500641
Adiponectin (human) EIA Kit (HS) - Item No. 10007619
Adiponectin (mouse) EIA Kit - Item No. 10007620
FABP4 (human) EIA Kit - Item No. 10007614
FTO (intracellular; mouse) EIA Kit - Item No. 579060
Ghrelin (human acylated) EIA Kit - Item No. 10006306
Ghrelin (human unacylated) EIA Kit - Item No. 10008952
Ghrelin (rat acylated) EIA Kit - Item No. 10006307
Ghrelin (rat unacylated) EIA Kit - Item No. 10008953
Leptin (human) EIA Kit - Item No. 500010
Leptin (mouse/rat) EIA Kit - Item No. 10007609
Leptin Receptor (human) EIA Kit - Item No. 10007608
Nampt/Visfatin (human) EIA Kit - Item No. 579020
Nampt/Visfatin (intracellular; human) EIA Kit - Item No. 579030
Nampt/Visfatin (mouse/rat) EIA Kit - Item No. 579040
Nampt/Visfatin (intracellular; mouse/rat) EIA Kit - Item No. 579050
Resistin (human) EIA Kit - Item No. 10007610
Resistin (rat) EIA Kit - Item No. 10007612
Vaspin (human) EIA Kit - Item No. 579000

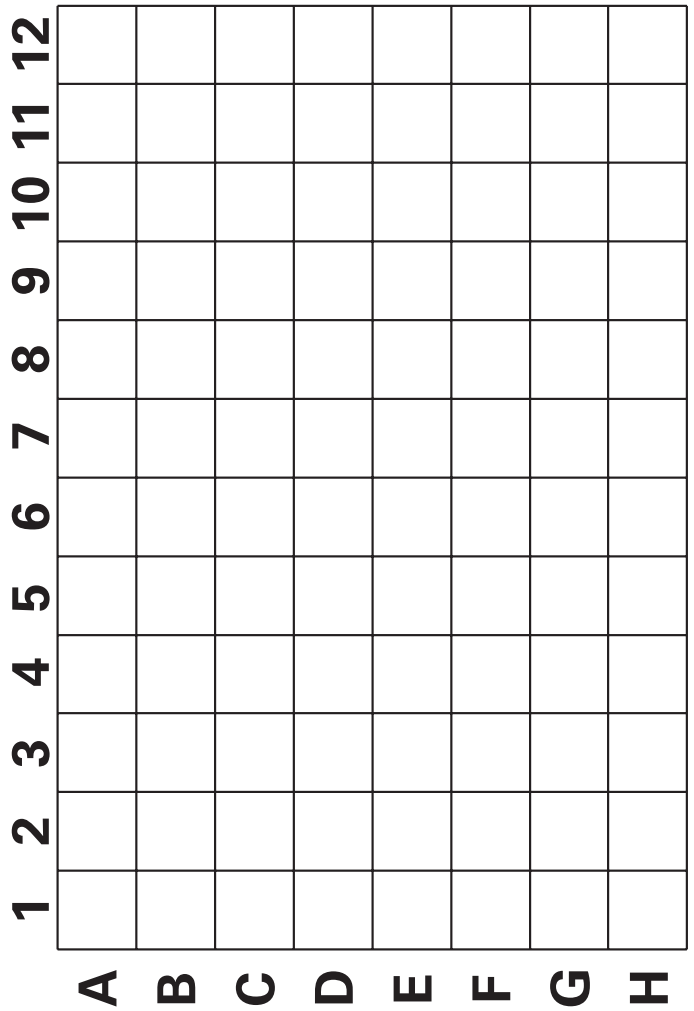
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

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