

KAMIYA BIOMEDICAL COMPANY

Estrone EIA kit

**For the quantitative determination of estrone in
dried fecal extracts, urine and tissue culture media**

Cat. No. KT-720

For Research Use Only.

PRODUCT INFORMATION

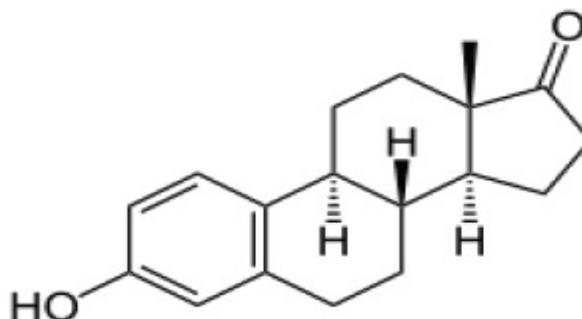
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BACKGROUND

Estrone, $C_{18}H_{22}O_2$, also known as E1 or osterone (3-hydroxy-1,3,5(10)-estratrien-17-one) is a C-18 steroid hormone. Estrone is one of the three naturally occurring estrogens, the others being estradiol and estriol. Estrone is produced primarily from androstenedione originating from the gonads or the adrenal cortex and from estradiol by 17-hydroxysteroid dehydrogenase. Androstenedione is also converted into estrone by aromatase (CYP19) to estrone and is expressed in stromal and carcinoma or parenchymal components of breast cancer tissue. Estrone concentrations in premenopausal mammals fluctuate according to the menstrual cycle. In premenopausal women, more than 50% of the estrone is secreted by the ovaries. In prepubertal children, men and non-supplemented postmenopausal women the major portion of estrone is derived from peripheral tissue conversion of androstenedione. Interconversion of estrone and estradiol also occurs in peripheral tissue. In humans, during the follicular phase of the menstrual cycle estrone levels increase slightly. The production of estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 the estrone levels will be low. A second peak occurs at around day 21 of the cycle and if fertilization does not occur, then the production of estrone decreases.

Estrone



PRINCIPLE

The Estrone Immunoassay kit is designed to quantitatively measure estrone present in extracted dried fecal samples, urine and tissue culture media samples. The kit is unique as it measures both non-conjugated estrone, estrone-3-sulfate and estrone 3-glucuronide in urine and fecal samples with almost equal affinity, allowing for non-invasive testing of this steroid.

Please read the complete kit insert before performing this assay. An estrone calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Calibrators or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An estrone-peroxidase conjugate is added to the calibrators and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to estrone to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound estrone-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the estrone in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

COMPONENTS

Coated Clear 96 Well Plates

Clear, break-apart 1 by 8 strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG.
1 Each

Estrone Calibrator

Estrone at 20,000 pg/mL in a special stabilizing solution.
125 μ L

Estrone Antibody

A rabbit polyclonal antibody specific for estrone.
3 mL

Estrone Conjugate

A estrone-peroxidase conjugate in a special stabilizing solution.
3 mL

Assay Buffer Concentrate

A 5X concentrate that should be diluted with deionized or distilled water.
28 mL

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.
30 mL

TMB Substrate

11 mL

Stop Solution

A 1M solution of hydrochloric acid. CAUSTIC.
5 mL

Plate Sealer

1 Each

STORAGE

All components of this kit should be stored at 4 °C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 µL.

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using.

Estrone is identical across all species and we expect this kit to measure estrone from all sources. The end user should evaluate recoveries of estrone in other sample matrices being tested.

SAMPLE PREPARATION

Dried Fecal Samples

The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Urine Samples

Urine samples should be diluted \geq with the provided Assay Buffer.

Tissue Culture Media

For measuring estrone in tissue culture media (TCM), samples should be read off a calibration curve generated in TCM. Samples may need to be diluted further in TCM. Using RPMI-1640 media we obtained 103% agreement of calibrators in TCM read off the Assay Buffer calibration curve.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine estrone concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

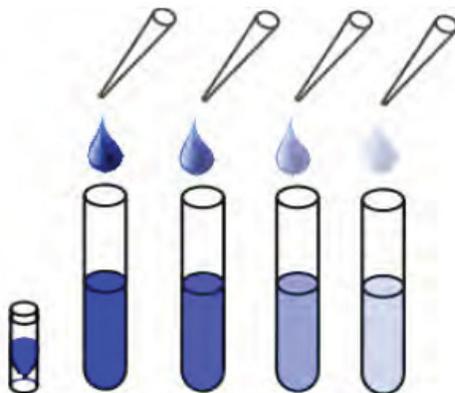
Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Calibrator Preparation

Label seven test tubes as #1 through #7. Pipet 450 μ L of Assay Buffer into tube #1 and 250 μ L into tubes #2 to #7. **The estrone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 μ L of the estrone stock solution to tube #1 and vortex completely. Take 250 μ L of the estrone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of estrone in tubes 1 through 7 will be 2,000, 1,000, 500, 250, 125, 62.5, and 31.25 pg/mL.

Use all Calibrators within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (μL)	450	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (μL)	50	250	250	250	250	250	250
Final Conc (pg/mL)	2,000	1,000	500	250	125	62.5	31.25

ASSAY PROTOCOL

1. Use the plate layout sheet on the back page to aid in proper sample and calibrator identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4°C.
2. Pipet 50 μL of samples or calibrators into wells in the plate.
3. Pipet 75 μL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 μL of Assay Buffer into wells to act as maximum binding wells (B_0 or 0 pg/mL).
5. Add 25 μL of the Estrone Conjugate to each well using a repeater pipet.
6. Add 25 μL of the Estrone Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken OD signal will be approximately 24% lower. %B/ B_0 will not be effected.
8. Aspirate the plate and wash each well 4 times with 300 μL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 μL of the TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 μL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate estrone concentration for each sample.

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/ B_0 curve, should be multiplied by the dilution factor to obtain neat sample values.

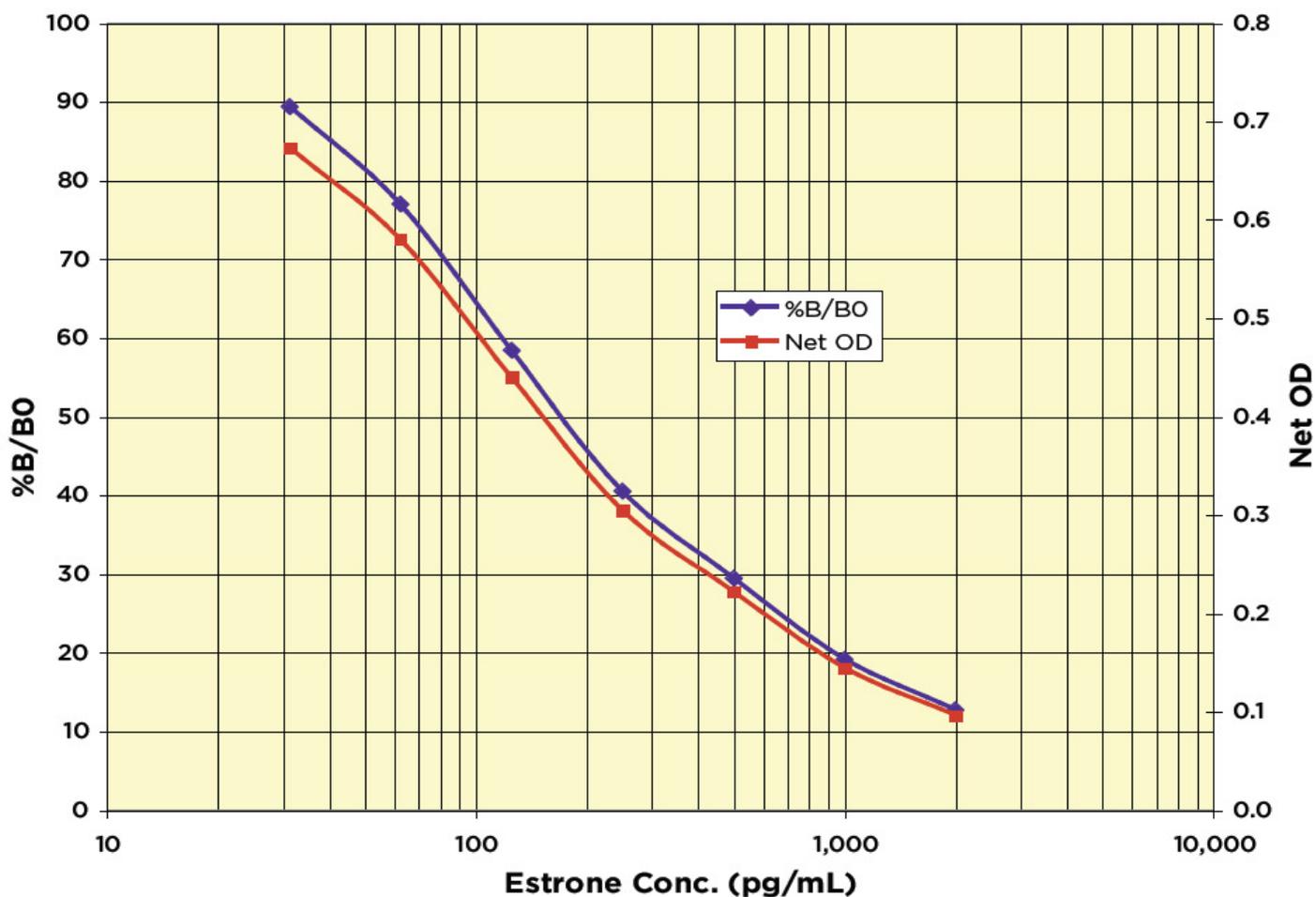
TYPICAL DATA

Sample	Mean OD	Net OD	% B/BO	Estrone Conc. (pg/mL)
NSB	0.048	0	-	-
Standard 1	0.144	0.096	12.7	2,000
Standard 2	0.192	0.144	19.1	1,000
Standard 3	0.270	0.222	29.5	500
Standard 4	0.353	0.305	40.5	250
Standard 5	0.488	0.440	58.4	125
Standard 6	0.628	0.580	77.0	62.5
Standard 7	0.721	0.673	89.4	31.25
BO	0.801	0.753	100.0	0
Sample 1	0.342	0.294	39.0	283.7
Sample 2	0.611	0.563	74.7	65.6

Always run your own calibration curve for calculation of results. Do not use this data.

Conversion Factor: 100 pg/mL of estrone is equivalent to 369.9 pM.

Typical Calibration Curves



Always run your own calibration curves for calculation of results. Do not use these data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and calibrator #7. The detection limit was determined at two (2) standard deviations from the B0 along the calibration curve.

Sensitivity was determined as 22.4 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero calibrator and a low concentration human sample.

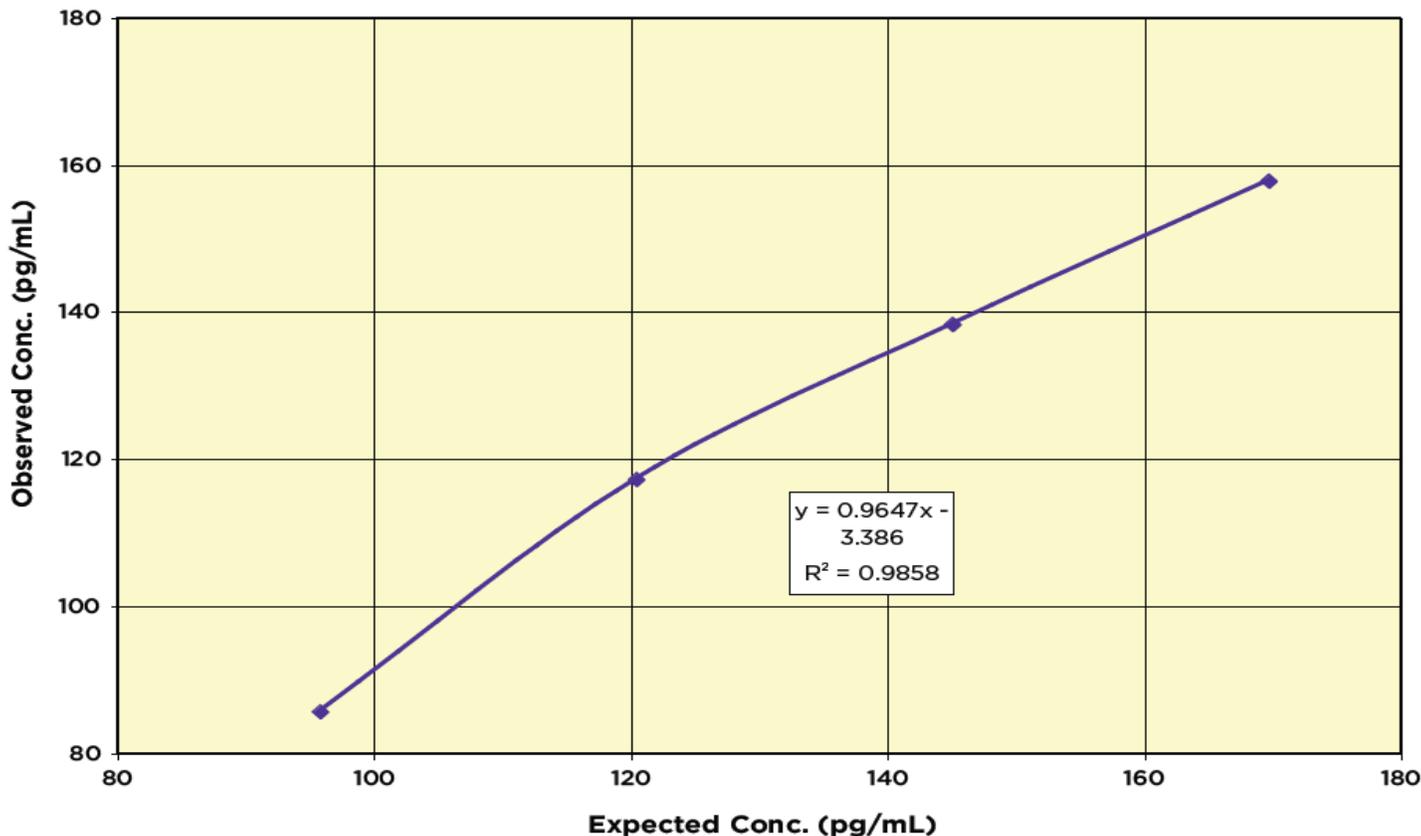
Limit of Detection was determined as 28.2 pg/mL

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted estrone level of 71.2 pg/mL and one with a higher diluted level of 194.4 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	157.8	169.8	93.0
60%	40%	138.3	145.1	95.3
40%	60%	117.2	120.5	97.3
20%	80%	85.6	95.8	89.3
Mean Recovery				93.7%

Linearity



Intra Assay Precision

Three urine samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Estrone concentrations were:

Sample	Estrone Conc. (pg/mL)	%CV
1	278.1	4.0
2	165.7	4.4
3	65.7	5.7

Inter Assay Precision

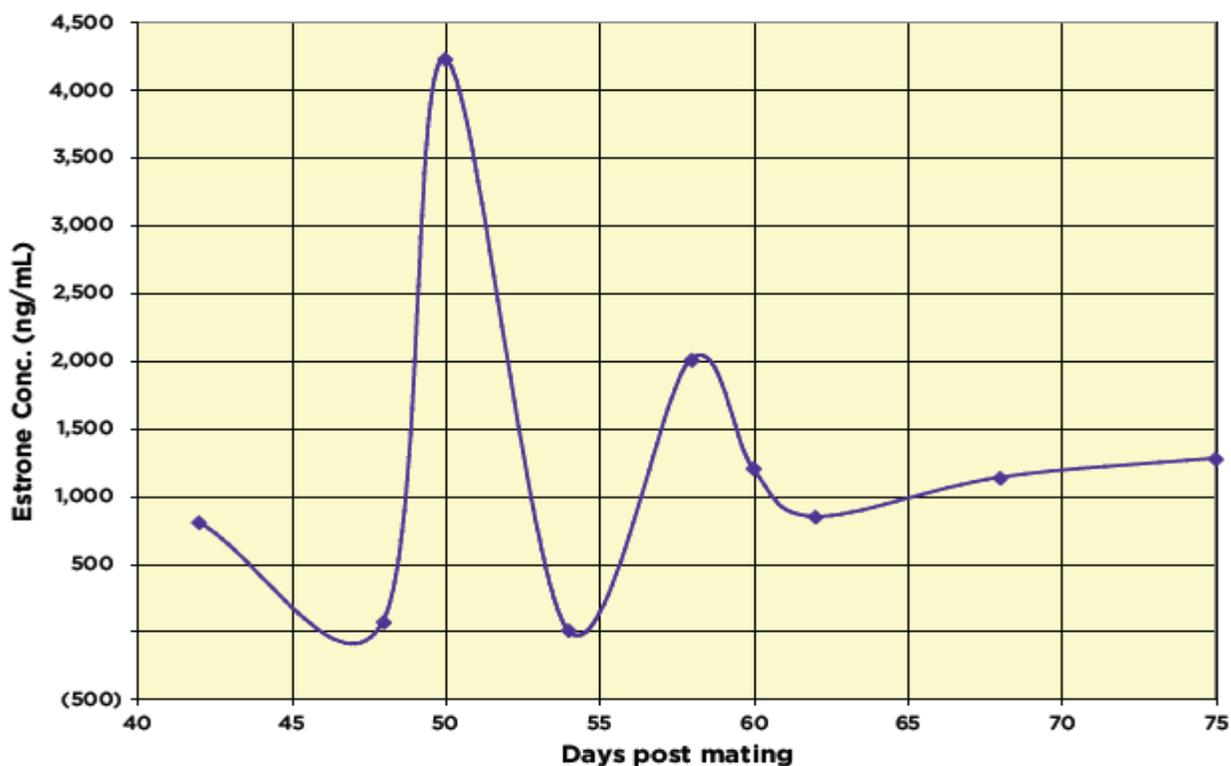
Three urine samples were diluted with Assay Buffer and run in duplicates in twelve assays run over multiple days by three operators. The mean and precision of the calculated Estrone concentrations were:

Sample	Estrone Conc. (pg/mL)	%CV
1	277.5	4.2
2	168.2	4.9
3	64.2	7.3

SAMPLE VALUES

Six human urine samples were tested in the assay. Adjusted neat concentrations of estrone ranged from 1.28 to 26.3 ng/mL, with an average value of 8,935 ng/mL.

Timed urine samples from a pregnant Maned Wolf over a 33-day period were tested in the assay. Day 75 was post birth.

Maned Wolf Urine

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Estrone	100%
Estrone 3-glucuronide	112%
Estrone 3-sulfate	65.5%
Estradiol	5.0%
Estradiol-3-sulfate	<0.1%
Estriol	<0.1%
Progesterone	<0.1%
Pregnandiol	<0.1%
Cortisol	< 0.1%
Androsterone	< 0.1%

FOR RESEARCH USE ONLY**KAMIYA BIOMEDICAL COMPANY**

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