StressMarq Biosciences INC.

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StressXpress®

Corticosterone EIA Kit

Catalog# SKT-205 (96-Well Kit)

Colorimetric detection of corticosterone

EAGLE BIOSCIENCES
EAGLEBIO.COM | INFO@EAGLEBIO.COM
P: 617-419-2019 | F: 617-419-1110

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-205A	Coated Clear 96 Well Plates	1 Each	A clear plastic microtiter plate coated with donkey anti-sheep IgG.
SKC-205B	Corticosterone Standard	125µL	Corticosterone at 100,000 pg/mL in a special stabilizing solution.
SKC-205C	StressXpress® Corticosterone Antibody	3mL	A sheep polyclonal antibody specific for corticosterone.
SKC-205D	StressXpress® Corticosterone Conjugate	3mL	A corticosterone-peroxidase conjugate in a special stabilizing solution.
SKC-205E	Assay Buffer	28 mL	Ready-to-use Assay Buffer.
SKC-205F	Dissociation Reagent	1 mL	Dissociation Reagent is to be used only with Serum and Plasma samples.
SKC-205G	Wash Buffer Concentrate	30 mL	A 20X concentrate that should be diluted with deionized or distilled water.
SKC-205H	TMB Substrate	11 mL	-
SKC-205I	Stop Solution	5 mL	A 1M solution of hydrochloric acid. CAUSTIC.
SKC-205J	Plate Sealer	1 Each	-

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete booklet 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 on page 8.

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

Storage

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

Materials Needed But Not Supplied

- Distilled or deionized water.
- Repeater pipet with disposable tips capable of dispensing 25 µL, 50 µL and 100 µL.
- 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.

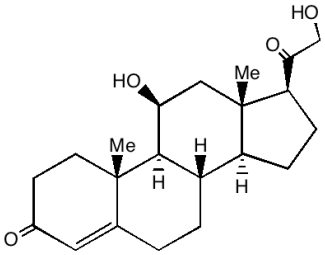
Please read this booklet completely prior to using the product.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Corticosterone ($C_{21}H_{30}O_4$, Kendall's Compound 'B') is a glucocorticoid secreted by the cortex of the adrenal gland. Corticosterone is produced in response to stimulation of the adrenal cortex by ACTH and is the precursor of aldosterone. Corticosterone is a major indicator of stress and is the major stress steroid produced in non-human mammals. Studies involving corticosterone and levels of stress include impairment of long term memory retrieval[1], chronic corticosterone elevation due to dietary restrictions[2] and in response to burn injuries[3]. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns[4,5].



Assay Principle

The Corticosterone StressXpress® EIA kit is designed to quantitatively measure Corticosterone present in serum, plasma, urine, extracted dried fecal samples, and tissue culture media samples. Please read the complete kit booklet before performing this assay. This kit measures total corticosterone in serum and plasma and in extracted fecal samples.

A corticosterone stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve. We provide protocols on page 8 to prepare assay standards from 5,000 to 78.125 pg/mL or from 10,000 to 78.125 pg/mL. Please choose the standard range that fits your sample concentrations most appropriately.

Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture sheep antibodies. A corticosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to corticosterone to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound corticosterone-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 corticosterone in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Serum, EDTA and Heparin Plasma, Urine, Dried Fecal Extracts, and Tissue Culture Media

This assay has been validated for serum, EDTA and heparin plasma, urine samples and for tissue culture samples. It has also been validated for dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit. Corticosterone is identical across all species and we expect this kit may measure corticosterone from sources other than human. The end user should evaluate recoveries of corticosterone in other samples being tested.

Sample Preparation

Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total corticosterone concentration in serum or plasma. Dissociation Reagent is to be used only with Serum and Plasma samples.

Serum and Plasma Samples

Allow the Dissociation Reagent to warm completely to Room Temperature before use. We suggest pipetting 5 µL of Dissociation Reagent into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the Dissociation Reagent in the tube, vortex gently and incubate at room temperature for 5 minutes or longer. Dilute with 490 µL of supplied Assay Buffer. This 1:100 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be ≥ 1:100.
NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

Urine Samples

Urine samples should be diluted ≥ 1:20 with the supplied Assay Buffer prior running in the assay. Please see our Creatinine Urinary Detection Kit, SKT-200,

for assays to measure urine creatinine which can be used to allow normalization of corticosterone in a random urine specimen.

Dried Fecal Samples

We have a detailed Extraction Protocol available on this product's website page. The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Tissue Culture Media

For measuring corticosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying.

Reagent Preparation

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

Wash Buffer

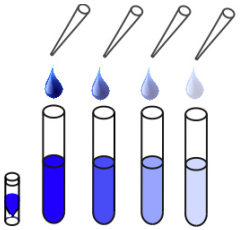
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable for 3 months at room temperature.

Standard Preparation

Label test tubes as #1 through #8. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #8. The corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add

50 µL of the corticosterone stock solution to tube #1 and vortex completely. Take 250 µL of the corticosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of corticosterone in tubes 1 through 8 will be 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.25, and 78.125 pg/mL.

Use all Standards within 2 hour of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8
Assay Buffer (µL)	450	250	250	250	250	250	250	250
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Volume of Addition (µL)	50	250	250	250	250	250	250	250
Final Concentration (pg/mL)	10,000	5,000	2,500	1,250	625	312.5	156.25	78.125

Assay Protocol

1. Use the plate layout sheet on page 21 to aid in proper sample and standard 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 standards 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 (Bo or 0 pg/mL).
5. Add 25 µL of the StressXpress® Corticosterone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the StressXpress® Corticosterone 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 1 hour. If the plate is not shaken signals bound will be approximately 45% lower.
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 or a multichannel 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 corticosterone concentration for each sample.

Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard 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/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

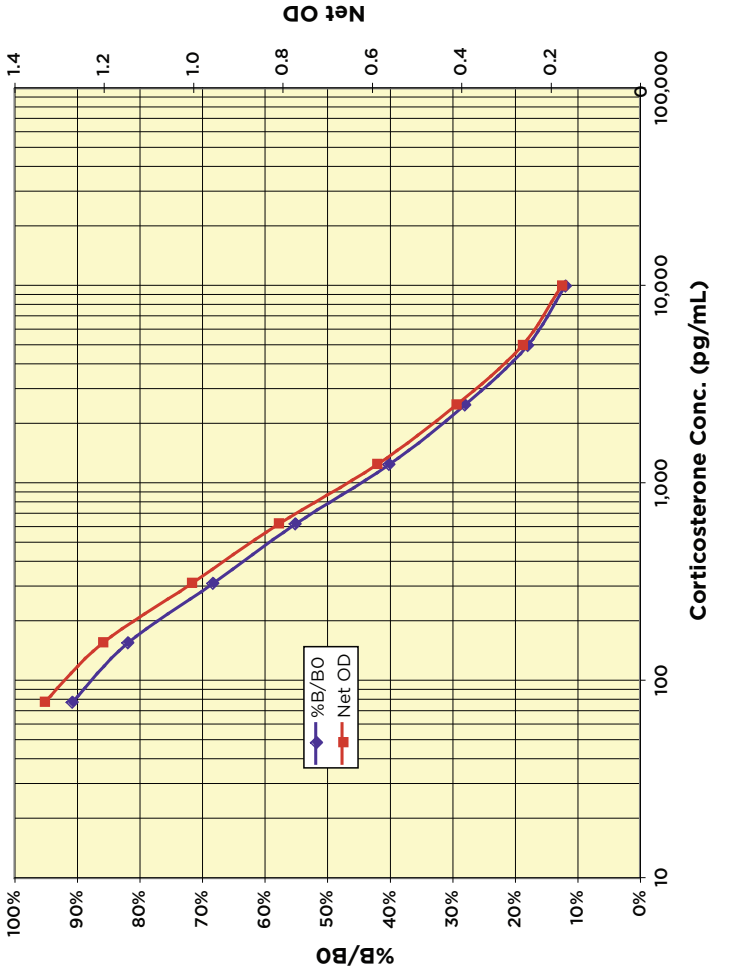
Typical Data

Sample	Mean OD	Net OD	% B/B0	Corticosterone Concentration (pg/mL)
NSB	0.067	0	-	-
Standard 1	0.242	0.175	11.9	10,000
Standard 2	0.330	0.263	17.9	5,000
Standard 3	0.478	0.411	28.0	2,500
Standard 4	0.656	0.589	40.1	1,250
Standard 5	0.876	0.809	55.1	625
Standard 6	1.070	1.003	68.3	312.5
Standard 7	1.269	1.202	81.8	156.25
Standard 8	1.400	1.333	90.7	78.125
B0	1.536	1.469	100.0	0
Sample 1	0.442	0.375	25.5	2,372
Sample 2	1.109	1.042	70.9	172.9

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

Conversion Factor: 100 pg/mL of corticosterone is equivalent to 288.6 pM.

Typical Normal Range Standard Curves



Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.
Sensitivity was determined as 18.6 pg/mL.

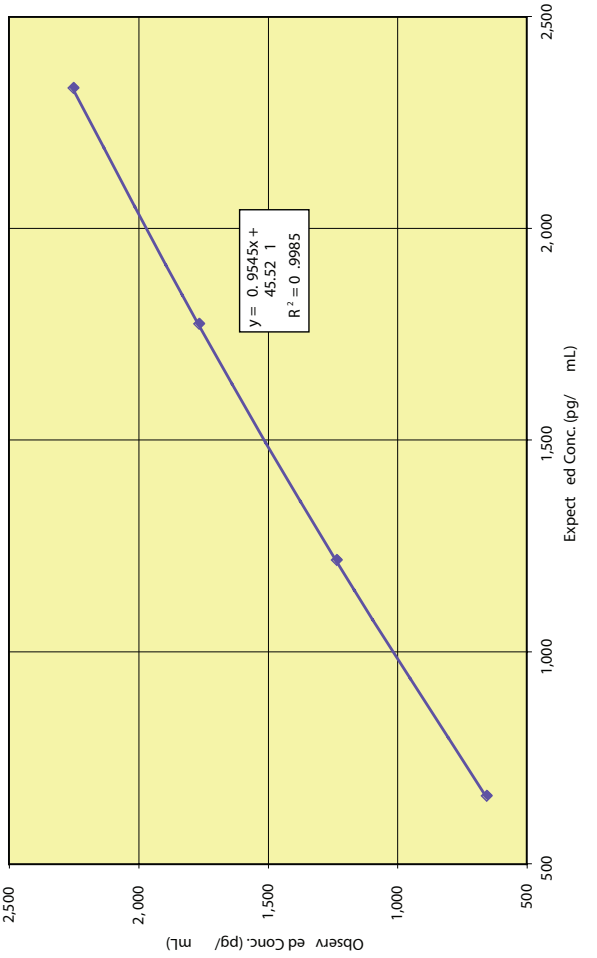
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.
Limit of Detection was determined as 16.9 pg/mL

Linearity

Linearity was determined by taking two serum samples treated with Dissociation Reagent and diluted 1:50 with Assay Buffer, one with a low diluted corticosterone level of 104.6 pg/mL and one with a higher diluted level of 2,890.5 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Serum	High Serum	Observed Concentration (pg/mL)	Expected Concentration (pg/mL)	% Recovery
100%	0%	104.6	--	--
80%	20%	654.0	661.8	98.8
60%	40%	1,232.3	1,219.0	101.1
40%	60%	1,763.9	1,776.1	99.3
20%	80%	2,249.5	2,333.3	96.4
0%	100%	2,890.5	--	--
			Mean Recovery	98.9%

Linearity


y = 0. 9545x + 45.52 1
R 2 = 0 .9985
500
1,000
1,500
2, 000
2,500
Expect ed Conc. (pg/ mL)
Observ ed Conc. (pg/ mL)

Intra Assay Precision

Four human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Corticosterone concentrations were:

Sample	Corticosterone Concentration (pg/mL)	%CV
1	2,460.6	6.3
2	601.5	6.5
3	371.6	3.1
4	259.0	4.8

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in fourteen assays run over multiple days by four operators. The mean and precision of the calculated Corticosterone concentrations were:

Sample	Corticosterone Conc. (pg/mL)	%CV
1	2,618.3	7.5
2	630.1	6.4
3	267.9	9.9

Sample Values

Six random mammalian serum and plasma samples were tested in the assay. Neat sample values ranged from 0.87 to 38.5 µg/dL with an average for the human samples of 1.56 µg/dL. The normal reference range for serum corticosterone is 0.13-2.3 µg/dL[6].

Dried fecal samples were processed as described on page 7 and run in the assay. Samples kindly donated by Dr. J. Williams at the Indianapolis Zoo, which included Amur Tiger, Giraffe, Kudu, Lion, Reeves Muntjac, White Handed Gibbon, White Rhino, and Zebra, were tested and corticosterone values obtained ranged from 7.85 to 81.6 pg/mg dried fecal material.

Palme and Möestl and colleagues have shown that radiolabeled administered glucocorticoids are excreted in differing amounts in urine and feces[7] across species, with fecal excretion ranging from 7% of administered cortisol in the pig to 82% in the cat[8-10]. Palme has also shown that the peak of fecal glucocorticoid concentrations occur at 12 hours for sheep, but takes 48 hours to peak in pigs. It is therefore necessary to evaluate the timing and relative fecal or urine excretion of glucocorticoids for each species.

Cross Reactivity

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

Steroid	Cross Reactivity (%)
Corticosterone	100%
Desoxycorticosterone	12.30%
Tetrahydrocorticosterone	0.76%
Aldosterone	0.62%
Cortisol	0.38%
Progesterone	0.24%
Dexamethasone	0.12%
Corticosterone-21-Hemisuccinate	<0.1%
Cortisone	< 0.08%
Estradiol	< 0.08%

References

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2. Kitaysky AS, Kitaiskaia EV, Wingfield JC, Piatt JF. "Dietary restrictions causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks." J. Comp. Physiol, 2001; 171: 701-709.
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4. Krame, KM. and Sothern RB. "Circadian characteristics of corticosterone secretion in red-backed voles (Clethrionomys gapperi)." Chronobiol. Int., 2001; 18(6): 933-945.
5. Vazquez-Palacios G, et al, "Further definition of the effect of corticosterone on the sleep-wake pattern in the male rat." Pharmacol. Biochem Behavior, 2001: 70(2-3): 305-310.
6. Tietz, NW, In "Textbook of Clinical Chemistry", WB Saunders, 1986.
7. Möstl, E., et al, Vet. Res. Commun. "Measurement of Cortisol Metabolites in Faeces or Ruminants." 2002, 26:127-139.
8. Palme, R., et al, Animal Reprod. Sci., "Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock." 1996, 43:43-63.
9. Teskey-Gerstl, A., et al, J. Comp. Physiol. B, "Excretion of corticosteroids in urine and faeces of hares (Lepus europaeus)." 2000, 170: 163-168.
10. Schatz, S. and Palme, R., Vet. Res. Commun., Measurement of Faecal Cortisol Metabolites in Cats and Dogs: A Non-Invasive Method for Evaluating Adrenocortical Function.", 2001, 25:271-287.

Warranty and Limitation of Remedy

StressMarq Biosciences Inc. 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. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq'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 StressMarq 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 Refund Policy located on our website and in our catalog.

Contact Information

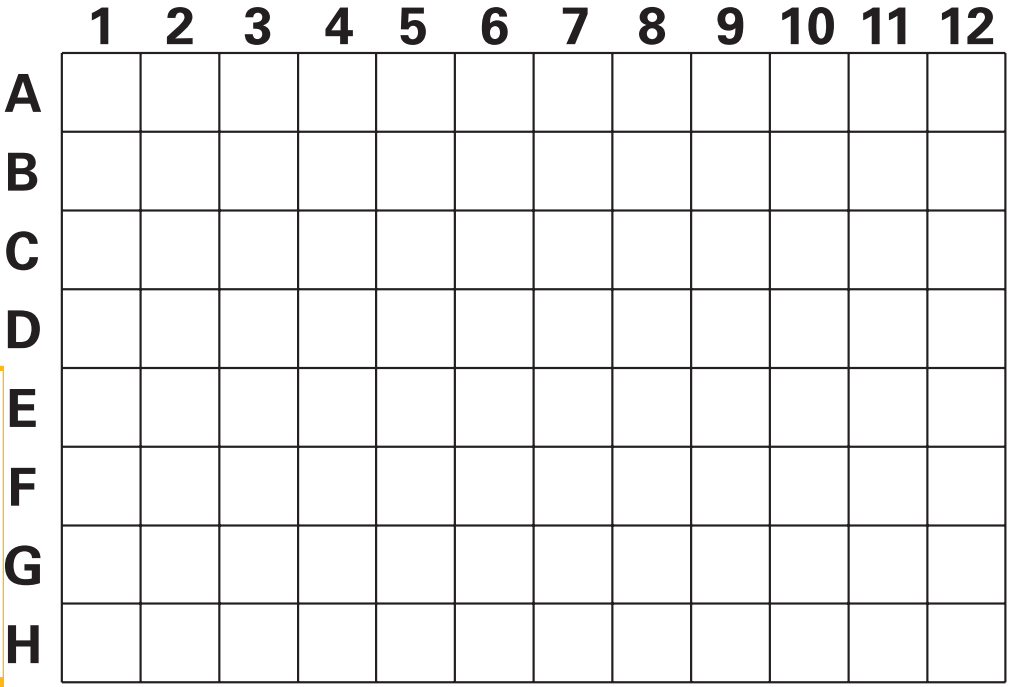
Phone: 250-294-9065

Fax: 250-294-9025

E-Mail: techsupport@stressmarq.com

Hours: M-F 9:00 AM to 5:00 PM PST

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).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