StressMarq Biosciences INC.

Discovery through partnership | Excellence through quality

StressXpress®

Cortisol EIA Kit

Catalog# SKT-201 (96-Well Kit)

Quantitative colorimetric detection of cortisol

TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Precautions
4 Storage
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
6 Assay Overview

PRE-ASSAY PREPARATION
7 Sample Types
7 Sample Preparation
9 Reagent Preparation

ASSAY PROTOCOL
11 Assay Protocol

ANALYSIS
12 Calculation of Results
12 Typical Data
13 Validation Data
15 Sample Values
16 Cross Reactivity

RESOURCES
17 References
18 Warranty and Contact Information
19 Plate Template
20 Notes

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-201A	Clear Coated 96 Well Plate	1 Plate	Coated with goat anti-mouse IgG.
SKC-201B	Cortisol Standard	125µL	Cortisol at 32,000 pg/mL in a special stabilizing solution.
SKC-201C	StressXpress® Cortisol Antibody	3mL	A mouse monoclonal antibody specific for cortisol (Green).
SKC-201D	StressXpress® Cortisol Conjugate	50µL	A cortisol-peroxidase conjugate in a special stabilizing solution. 100X Concentrate
SKC-201E	StressXpress® Cortisol Conjugate Diluent	5mL	A proprietary blend of proteins, buffers, detergents and dye (Purple)
SKC-201F	Assay Buffer	50mL	Ready to use Assay Buffer (Red)
SKC-201G	Dissociation Reagent	1mL	Dissociation Reagent is to be used only with Serum and Plasma samples. Allow to warm completely to Room Temperature prior to use.
SKC-201H	Wash Buffer Concentrate	50mL	A 10X concentrate that should be diluted with deionized or distilled water.
SKC-201I	TMB Substrate	11mL	-
SKC-201J	Stop Solution	5mL	A 0.36M solution of sulfuric acid. CAUSTIC.
SKC-201K	Plate Sealer	1Each	-

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.

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 9.

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 this kit, with the exception of Catalog No. SKC-201D, the Cortisol Conjugate and the Standard, Catalog No. SKC-201B, which must be stored at -20°C.

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.
- Orbital micro-plate shaker

Please read this booklet completely prior to using the product.

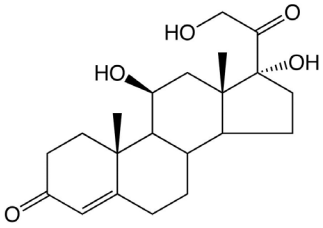
FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Cortisol, C21H30O5, (hydrocortisone) is the primary glucocorticoid produced and secreted by the adrenal cortex. It is often referred to as the "stress hormone" as it is involved in the response to stress and it affects blood pressure, blood sugar levels, and other actions of stress adaptation. Immunologically, cortisol functions as an important anti-inflammatory and plays a role in hypersensitivity, immunosuppression, and disease resistance1. In the metabolic aspect, cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization2. Production of cortisol follows an ACTH-dependent circadian rhythm, with a peak level in the morning and decreasing levels throughout the day. Most serum cortisol, all but about 4%, is bound to proteins including corticosteroid binding globulin and serum albumin1,3. Only free cortisol is available to most receptors and it is through these receptors that physiological processes are modulated. Abnormal cortisol levels are being evaluated for correlation with a variety of different conditions, such as prostate cancer4, depression5, and schizophrenia6. It is already known that abnormal levels of cortisol are involved in Cushing's Syndrome and Addision's disease7.



Assay Overview

The Cortisol EIA kit is designed to quantitatively measure cortisol present in dried fecal extracts, saliva, urine, serum, plasma and tissue culture media samples. Please read the complete kit booklet before performing this assay. This kit measures total cortisol in extracted samples and in serum and plasma and free cortisol in saliva and urine. A cortisol standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture mouse antibodies. A cortisol-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to cortisol to each well. After an 1 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound cortisol-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 450 nm wavelength. The concentration of the cortisol 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:
Dried Fecal Extracts, Saliva, Urine, Serum, EDTA and Heparin Plasma and Tissue Culture Media

Cortisol is identical across all species and we expect this kit may measure cortisol from sources other than human. The end user should evaluate recoveries of cortisol in other samples being tested.
Please note that samples containing mouse IgG may interfere with this assay.
This assay has been validated for saliva, urine, serum and EDTA and heparin plasma 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.

Sample Preparation

Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total cortisol concentration in serum or plasma. Dissociation Reagent is to be used only with Serum and Plasma samples. Free cortisol can be measured in saliva and urine samples as directed below.

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%.

Serum and Plasma Samples

Allow the Dissociation Reagent (DR) to warm completely to Room Temperature before use. We suggest pipetting 5 µL of DR into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the DR 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.

Saliva Samples

Saliva samples should be diluted ≥ 1:4 or greater with the supplied Assay Buffer prior running in the assay. See our Saliva Sample Handling Instructions available on this product's website page.

Urine Samples

Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay. Urinary cortisol normally ranges from 0.7-119 µg/gram8 of creatinine or approximately 100,000 to 1,000,000 pg/mL9 in 24 hour urine samples. Samples may need to be diluted substantially to read within the standard curve range.

Tissue Culture Media

For measuring cortisol 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 cortisol concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Cortisol Conjugate

1. Determine the amount of Cortisol Conjugate preparation required. For every immunoassay plate strip used (8 wells), prepare 300 µL of Cortisol Conjugate preparation.
2. Prepare Cortisol Conjugate preparation by diluting the Cortisol Conjugate 1/100 with Cortisol Conjugate Diluent (Purple). Mix well prior to use. For example, if 3mL of Cortisol Conjugate preparation is required, dilute 30 µL of Cortisol Conjugate in 3mL of Cortisol Conjugate Diluent. *Store the reconstituted Cortisol Conjugate on ice and use within 2 hours. Store any unused Cortisol Conjugate (Concentrate) at -20°C.

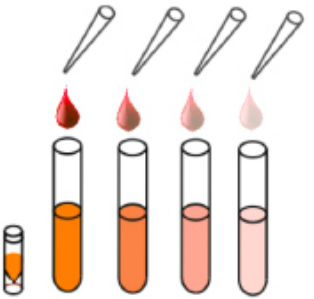
Wash Buffer

Dilute Wash Buffer Concentrate 1:10 by adding one part of the concentrate to nine parts of deionized water. Once diluted this is stable at room temperature for 3 months at room temperature.

Standard Preparation

Label six test tubes as #1 through #6. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #6. The cortisol stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the cortisol stock solution to tube #1 and vortex completely. Take 250 µL of the cortisol solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of cortisol in tubes 1 through 6 will be 3,200, 1,600, 800, 400, 200, and 100 pg/mL.

Use all Standards within 2 hour of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Assay Buffer Volume (µL)	450	250	250	250	250	250
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Volume of Addition (µL)	50	250	250	250	250	250
Final Concentration (pg/mL)	3,200	1,600	800	400	200	100

Assay Protocol

1. Use the plate layout sheet on page 19 to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to 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® Cortisol Conjugate to each well using a repeater pipet.
6. Add 25 µL of the StressXpress® Cortisol 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 at 600rpm* critical step for proper assay function *
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 with 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 cortisol concentration for each sample.

ANALYSIS

Calculation of Results

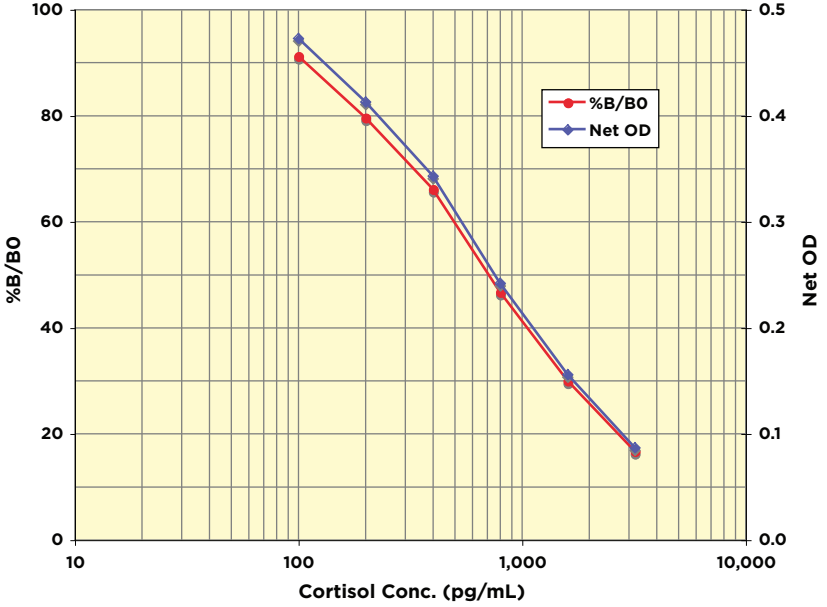
Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

Sample	Mean OD	Net OD	% B/B0	Cortisol Concentration (pg/mL)
NSB	0.044	0	-	-
Standard 1	0.131	0.087	16.7	3,200
Standard 2	0.200	0.156	30.1	1,600
Standard 3	0.286	0.242	46.7	800
Standard 4	0.387	0.343	66.2	400
Standard 5	0.457	0.413	79.7	200
Standard 6	0.517	0.473	91.3	100
B0	0.562	0.518	100.0	0
Sample 1	0.137	0.093	18.0	2974.9
Sample 2	0.478	0.434	83.8	163.9

Always run your own standard curve for calculation of results.
Do not use this data.
Conversion Factor: 100 pg/mL of cortisol is equivalent to 275.9 pM.

Typical 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 #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

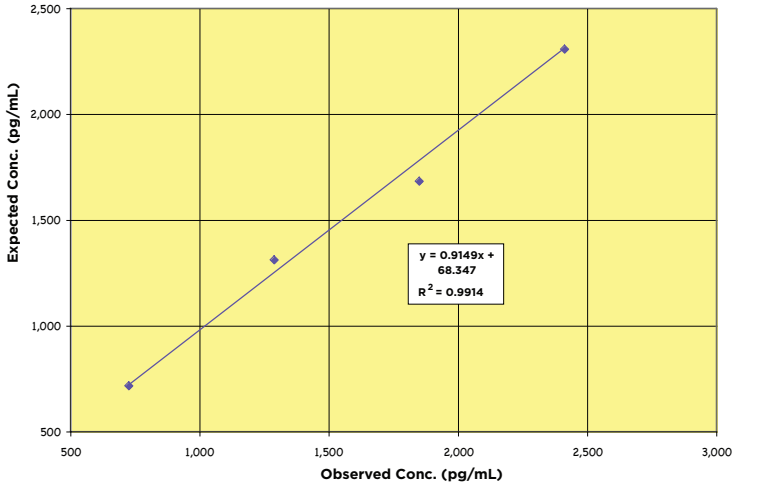
Sensitivity was determined as 30.3 pg/mL.

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 55.4 pg/mL

Linearity

Linearity was determined by taking two human urine samples diluted 1:140, one with a low diluted cortisol level of 163.9 pg/mL and one with a higher diluted level of 2,974.9 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 Urine	High Urine	Observed Concentration (pg/mL)	Expected Concentration (pg/mL)	% Recovery
100%	0%	163.9	--	--
80%	20%	715.7	726.1	98.6
60%	40%	1,311.5	1,288.3	101.8
40%	60%	1,683.3	1,850.5	91.0
20%	80%	2,306.3	2,412.7	95.6
0%	100%	2,974.9	--	--
			Mean Recovery	96.7%



Intra Assay Precision

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

Sample	Cortisol Concentration (pg/mL)	%CV
1	1,174.3	6.0
2	475.9	5.6
3	177.4	14.7

Inter Assay Precision

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

Sample	Cortisol Concentration (pg/mL)	%CV
1	1,188.1	7.2
2	508.7	6.3
3	199.7	10.9

Sample Values

Six random human serum and plasma samples were tested in the assay. Neat sample values ranged from 8.5 to 23.8 µg/dL with an average of 12.2 µg/dL. The normal reference range for serum cortisol is 3-23 µg/dL9. Four random human urine samples were tested in the assay. Neat sample values ranged from 98.1 to 304.9 µg/g creatinine with an average of 159.8 µg/g creatinine. Creatinine levels were determined using the StressXpress® Creatinine Urinary Detection Kit, SKT-200.

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 cortisol values obtained ranged from 2.48 to 27.22 pg/mg dried fecal material.

Palme and Möestl and colleagues have shown that radiolabeled administered cortisol is excreted in differing amounts in urine and feces10 across species, with fecal excretion ranging from 7% of administered cortisol in the pig to 82% in the cat11,12, 13. Palme has also shown that the peak of fecal cortisol 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 (%)
Cortisol	100%
11-Deoxycorticosterone	<0.1%
Prednisolone (1-Dehydrocortisol)	5.6%
Corticosterone	0.6%
11-Hydroxyprogesterone	<0.1%
Progesterone	<0.1%
Estradiol	<0.1%
Danazol	<0.01%

References

1. E. Friess, et al., Eur J Clin Invest, 2000, 30, Suppl 3:46-50.
2. Freeman, Scott, 2002. Biological Science. Prentice Hall; 2nd Pkg edition (December 30, 2004).
3. C. Longscope., J. Endocrinology, 1996, , Suppl S125-S127.
4. J. Herbert, Lancet, 1995 345, 1193-1194.
5. A. Michael, et al., Biol. Psychiatry, 2000, 48, 989-95.
6. C.R. Dequet and D.J. Wallace, Current Opin. Ivest. Drugs, 2001, 8, 1045-53.
7. W.M. Jeffries, Med. Hypotheses, 1998, 51, 114-4.
8. "82920 Clinical: Cortisol, Free, Random, Urine." Mayo Medical Laboratories: Reference Laboratory services for hospitals worldwide. Web. 04 Aug. 2009. <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/82920>.
9. Tietz, NW, In "Textbook of Clinical Chemistry", WB Saunders, 1986.
10. Möstl, E., et al, Vet. Res. Commun. "Measurement of Cortisol Metabolites in Faeces or Ruminants." 2002, 26:127-139.
11. Palme, R., et al, Animal Reprod. Sci., "Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock." 1996, 43:43-63.
12. Teskey-Gerstl, A., et al, J. Comp. Physiol. B, "Excretion of corticosteroids in urine and faeces of hares (Lepus europaeus)." 2000, 170: 163-168.
13. 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

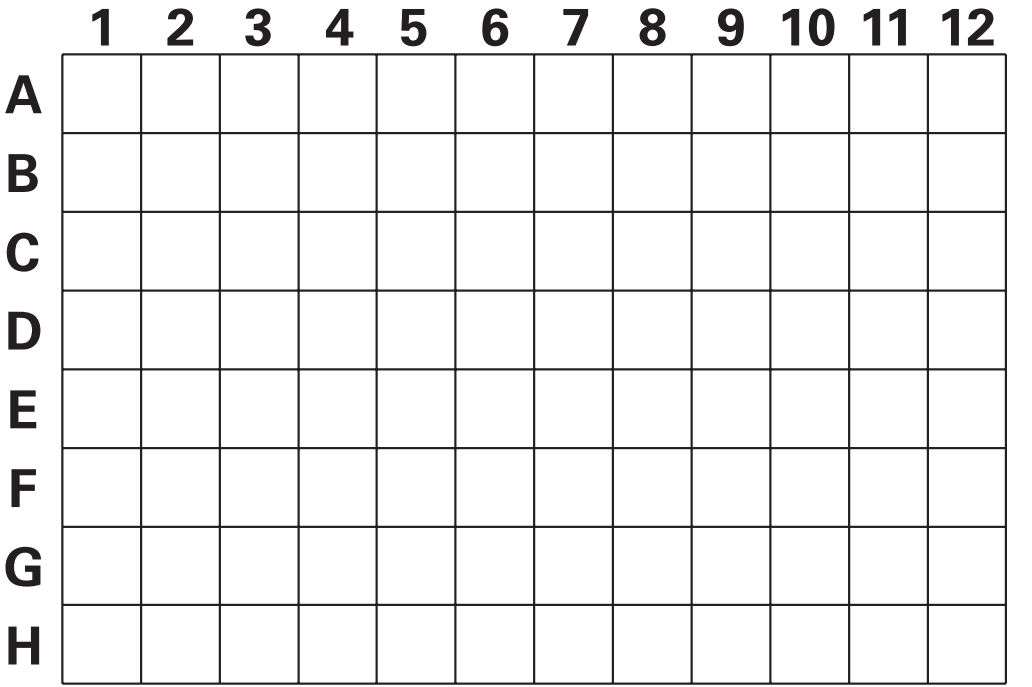
Phone: 250-294-9065

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).


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

NOTES

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from StressMarq Biosciences Inc. ©08/28/2010, StressMarq Biosciences Inc., Victoria, BC Canada, All rights reserved. Printed in Canada.