StressMarq Biosciences INC.

Discovery through partnership | Excellence through quality

Alpha Synuclein Oligomer ELISA Kit

Catalog# SKT-143-96 (96-Well Kit)

Colorimetric detection of Alpha Synuclein Oligomer

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GENERAL INFORMATION

Materials Supplied

Catalog No.	Item	Quantity/Size
SKC-143A	Anti-Oligomeric Alpha Synuclein Immunoassay Plate	1 Plate
SKC-143B	Recombinant Oligomeric Alpha Synuclein Standard	2 Vials
SKC-143C	Standard and Sample Diluent (Red)	1 Bottle/50mL
SKC-143D	10X Wash Buffer Concentrate	1 Bottle/ 100mL
SKC-141E	Anti-Oligomeric Alpha Synuclein Biotinylated Antibody Concentrate	1 Vial/ 150µL
SKC-141F	Antibody Diluent Buffer (Green)	1 Bottle/13mL
SKC-141G	Streptavidin: HRP Concentrate	1 Vial/ 150µL
SKC-141H	Streptavidin: HRP Diluent (Purple)	1 Bottle/ 13mL
SKC-141I	TMB Substrate	1 Bottle/ 13mL
SKC-141J	Stop Solution	1 Bottle/ 13mL

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

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with StressMarq Biosciences Inc.'s ELISA Kits. 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:	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).

Storage and Stability

Storage and Stability All reagents with the exception of the Plate, Antibody, Standard and Streptavidin:HRP Concentrate are stable as supplied at 4°C, the Plate, Antibody, Standard and Streptavidin HRP Concentrate should be stored at -20°C. Unused wells should be resealed with desiccant in the foil pouch provided, and stored at -20°C until the kits expiry date.

Materials Needed But Not Supplied

- Ultra pure water
- Additional reagents and materials for cell lysate and tissue extract preparation, including protease inhibitors
- Precision pipettors, with disposable plastic tips
- Polypropylene or polyethylene tubes to prepare samples – do not use polystyrene, polycarbonate or glass tubes
- A container to prepare 1X Wash Buffer
- A wash bottle or an automated 96-well plate washer
- Disposable reagent reservoirs
- Shaking Plate Incubator
- A standard microtiter plate reader for measuring absorbance at 450 nm
- Adhesive plate sealers

Assay Precautions

- All ELISA reagents must be at room temperature (20-25°C) before use.
- Vigorous plate washing is essential.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
- Take care not to contaminate the TMB Substrate. Do not expose TMB Substrate solution to glass, foil, or metal. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.

INTRODUCTION

Background

Alpha-synuclein (αS) is a 140-amino acid, intrinsically disordered protein central to Parkinson's disease (PD) pathology. Its structure comprises three key regions: an amphipathic N-terminus that forms α-helices upon membrane binding[1,2,3] a central hydrophobic NAC domain (residues 61–95) crucial for α-sheet formation and aggregation[4,5] and an acidic, proline-rich C-terminus that remains flexible and interacts with various ligands[6,7]. In its native state, αS exists in a dynamic equilibrium between disordered monomers and helical tetramers[8,9]. However, under specific cellular conditions, it can misfold, with the NAC region driving the formation of α-sheet-rich oligomeric intermediates, which are considered the primary neurotoxic species[10]. This oligomerization is influenced by factors like pH, mutations (e.g., A30P, E46K, A53T)[4] and post-translational modifications such as phosphorylation at serine 129, a hallmark of pathological aggregates[11].

The pathological significance of alpha-synuclein oligomers is paramount in neurodegenerative synucleinopathies, particularly PD[12]. Mounting evidence indicates that these soluble oligomers, ranging from dimers to 150-mers with globular or annular morphologies[13] are significantly more toxic than mature amyloid fibrils[14,15]. They are the main drivers of neuronal dysfunction through mechanisms including membrane disruption, mitochondrial damage, and the induction of oxidative stress and neuroinflammation[16,17,18,19,20]. Critically, oligomers facilitate the prion-like spread of pathology between neurons, contributing to disease progression[21,22]. Their presence in cerebrospinal fluid (CSF) and peripheral tissues like the skin correlates with PD diagnosis and cognitive decline[23,24,25,26], making them a premier target for therapeutic intervention and a critical biomarker for early detection and monitoring of disease progression[27].

About This Assay

StressMarq Biosciences Inc.'s Molecular Signature™ Series ELISA Kit is for the detection of human and mouse Alpha Synuclein Oligomer in brain homogenates. Each kit contains sufficient components to quantitate the Alpha Synuclein Oligomer concentration in up to 40 samples, tested in duplicate.

ASSAY OVERVIEW

1. Prepare Standard and samples in Standard and Sample Diluent.
2. Add 100 µL of Standard or sample to appropriate wells.
3. Cover plate with Plate Sealer and incubate at Room Tempearture for 1 hour shaking at 600rpm.
4. Wash plate four times with 1X Wash Buffer.
5. Add 100 µL of Biotinylated Antibody Working Solution to each well.
6. Cover plate with Plate Sealer and incubate at Room Temperature for 1 hour shaking at 600rpm.
7. Wash plate four times with 1X Wash Buffer.
8. Add 100 µL of Streptavidin-HRP Working Solution to each well.
9. Cover plate with Plate Sealer and incubate at Room Temperature for 30 minutes, shaking at 600rpm.
10. Wash plate four times with 1X Wash Buffer.
11. Add 100 µL of TMB Substrate to each well.
12. Develop the plate in the dark at room temperature for 30 minutes.
13. Stop reaction by adding 100 µL of Stop Solution to each well.
14. Measure absorbance on a plate reader at 450 nm.

Sample Preparation

Extraction of Alpha Synuclein Oligomer Complexes from Brain Tissue

Extracting Alpha Synuclein Oligomer complexes from brain tissue can be done effectively using TBS extraction protocols.

Below is a suggested method for preparing brain samples to isolate oligomeric complexes.

Method Reference: Youmans KL et al. (2011), Amyloid-β42 Alters Apolipoprotein E Solubility in Brains of Mice with Five Familial AD Mutations. Journal of Neuroscience Methods, 196:51-59.

Homogenization and Initial Extraction

Take frozen brain hemispheres and homogenize them in TBS (Tris-buffered saline) containing a complete protease inhibitor mixture.

Centrifuge the homogenate at 16,000 x g for 30 minutes at 4°C.

Collect the supernatant, which is the TBS-soluble fraction, and store it at -80°C for future use.

Secondary Extraction

1. Resuspend the pellets in TBS plus 1% Triton X-100 (TBS-T) with protease inhibitors.
2. Sonicate the resuspended pellets for 5 minutes in a 4°C water bath.
3. Centrifuge the mixture again at 16,000 x g for 30 minutes at 4°C.
4. Collect the supernatant, which is the TBS-T-soluble fraction, and store it at -80°C. This fraction will be used as the sample source for the oligomeric ELISA assay.

Protein Concentration Measurement

Measure the total protein concentration of all samples using a detergent (ie. Triton X-100) compatible protein assay.

Normalize the ELISA results based on the protein concentration of each sample.

By following these steps, you can effectively extract and prepare Alpha Synuclein Oligomer complexes for analysis using an ELISA assay.

Sample Handling

- All biological materials should be handled as potentially
- hazardous. Follow universal precautions when handling and disposing of
- infectious agents.
- 100 µl of diluted sample is required per well.
- Samples must be assayed in duplicate each time the assay is performed.
- Samples should be frozen if not analyzed shortly after harvest. For long-term
- storage, aliquot and freeze samples. Avoid repeated freeze-thaw cycles when
- storing samples.
- If particulate is present in samples, centrifuge prior to analysis.
- If the integrity of the sample is of concern, make a note on the Plate Template
- and interpret results with caution

Sample Dilution

- Samples must first be diluted prior to testing.
- Suggested starting dilutions for samples:
- For Brain Homogenates dilute samples to 25 µg/mL protein concentration in Standard and Sample Diluent. For example, dilute 5 µL of a 1.25 mg/mL sample into 245 µL Standard and Sample Diluent. Mix well. *Note: If values for samples are not within the range of the standard curve, optimal sample dilutions need to be determined.*
- Prepare at least 250 µL of sample in Standard and Sample Diluent. Mix samples well prior to analysis.

Other Reagent Handling/ Preparation

Standard Preparation

1. Reconstitute standard vial with 1.0 mL of Standard and Sample Diluent for a concentration of 6,250 pg/mL. Mix well. This is the standard Stock Solution.
2. Transfer 160µL of the Standard Stock Solution to a tube containing 840 µL of Standard and Sample Diluent, resulting in a final concentration of 1,000 pg/mL.
3. Label seven (7) tubes, one for each additional standard curve point: 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL and 0 pg/mL.
4. Pipet 250 µl of Standard and Sample Diluent into each tube.
5. Serially dilute the 1000 pg/mL standard 1:1 with Standard and Sample Diluent.
6. Perform dilution by mixing 250 µL of the previous standard with 250 µL ofStandard and Sample Diluent. Continue until reaching the standard value of 15.6 pg/mL.
7. Use Standard and Sample Diluent only as the zero-standard value.

- **Note:** Keep the reconstituted Standard preparations cold on ice when not in use, freeze-thaw cycles disrupt the stability of the protein, therefore discard reconstituted preparations after use.

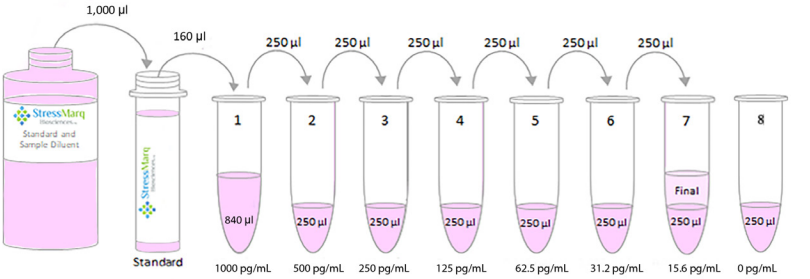

Figure 1. Preparation of the Alpha Synuclein Oligomer Standard

1X Wash Buffer Preparation

- Prepare 1X Wash buffer by diluting 10X Wash Buffer in ultra pure water. For example, if preparing 1 L of 1X Wash Buffer, dilute 100 mL of 10X Wash Buffer into 900 mL of ultra pure water. Mix well. Store reconstituted 1X Wash Buffer at 2-8ºC for up to one (1) month. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.

Note: The recommended wash volume per well is 200µL, the recommended wash frequency is 4 times per well during each wash cycle.

Biotinylated Antibody Working Solution Preparation

- Determine the amount of Biotinylated Antibody Working Solution required. For every strip-well used (8-wells), prepare 1 mL of Biotinylated Antibody Working Solution.
- Prepare Biotinylated Antibody Working Solution by diluting Biotinylated Antibody Concentrate 1:100 with Biotinylated Antibody Diluent. For example, if 12 mL of Biotinylated Antibody Working Solution is required (one whole plate), dilute 120 µL of Biotinylated Antibody Concentrate in 12 mL Biotinylated Antibody Diluent. Mix well prior to use.

Streptavidin-HRP Working Solution Preparation

- Determine the amount of Streptavidin-HRP Working Solution required. For every strip-well used (8-wells), prepare 1 mL of Streptavidin-HRP Working Solution.
- Prepare Streptavidin-HRP Working Solution by diluting Streptavidin-HRP Concentrate 1:100 with Streptavidin-HRP Diluent. For example, if 12 mL of Streptavidin-HRP Working Solution is required (one whole plate), dilute 120 µL of Streptavidin-HRP Concentrate in 12 mL Streptavidin-HRP Diluent. Mix well prior to use.

Performing the Assay

Sample Incubation

- Determine the number of strips required. Leave these strips in the plate frame. Place unused strips in the foil pouch with desiccant and seal tightly. Store unused strips at 2-8°C. After completing assay, keep the plate frame for additional assays.
- Use a Plate Template to record the locations of the standards and unknown samples within the wells.

1. Add 100 µL of appropriately diluted standards or samples to each well. Run each standard, sample, or blank in duplicate.
2. Carefully cover wells with a new adhesive plate cover. Incubate for one (1) hour at 37°C while shaking at 600rpm.
3. Carefully remove adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame.
2. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate plate contents from all wells and flood wells with 1X Wash Buffer. Repeat procedure three additional times for a total of FOUR washes. Additional washes may be necessary. Blot plate onto paper towels or other absorbent material. Take care to avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.

Biotinylated Antibody Incubation

- Prepare only the required amount of Biotinylated Antibody Working Solution for the number of strips being used.

1. Add 100 µL of Biotinylated Antibody Working Solution to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.
2. Carefully attach a new adhesive plate cover. Incubate plate for one (1) hour at 37°C while shaking at 600rpm..
3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

Streptavidin-HRP Incubation

- Prepare only the required amount of Streptavidin-HRP Working Solution for the number of strips being used.

1. Add 100 µL of Streptavidin-HRP Working Solution to each well containing standard, sample or blank.
2. Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at 37°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

TMB Substrate Incubation and Reaction Stop

- Only remove the required amount of TMB Substrate and Stop Solution for the number of strips being used.
- Do NOT use a glass pipette to measure the TMB Substrate solution. Do NOT cover the plate with aluminum foil or metalized mylar. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT!

1. Add 100 µL of TMB Substrate into each well.
2. Allow the enzymatic color reaction to develop at room temperature (20-25ºC) in the dark for 30 minutes. Do NOT cover plate with a plate sealer. The substrate reaction yields a blue solution.
3. After 30 minutes, stop the reaction by adding 100 µL of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should change from blue to yellow.

Absorbance Measurement

Note: Evaluate the plate within 30 minutes of stopping the reaction.

1. *Wipe underside of wells with a lint-free tissue.*
2. *Measure the absorbance on an ELISA plate reader set at 450 nm.*

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used.

Calculations

- Duplicate absorbance values should be within 10% of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10%.

1. Prepare a standard curve to determine the amount of Alpha Synuclein Oligomer in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding Alpha Synuclein Oligomer concentration on the horizontal (X) axis using graph paper or curve-fitting software.
2. Calculate the Alpha Synuclein Oligomer concentration in unknown samples using the prepared standard curve. Determine the amount of Alpha Synuclein Oligomer in each unknown sample by noting the Alpha Synuclein Oligomer concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.
3. Multiply the Alpha Synuclein Oligomer concentration obtained by the dilution factor to determine the amount of Alpha Synuclein Oligomer in the undiluted sample.

Performance Characteristics

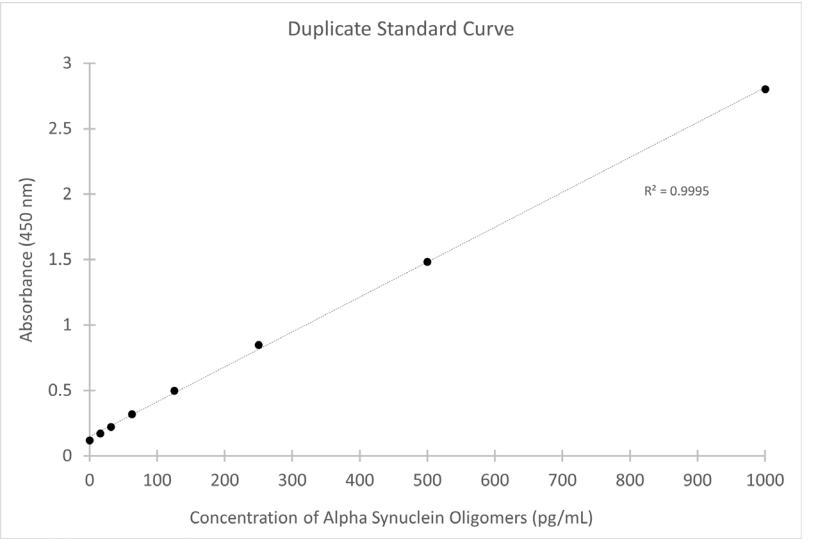

Figure 2. Typical Standard Curve for the Alpha Synuclein Oligomer ELISA Kit (SKT-143). Assay Type: Sandwich ELISA. Detection Method: Colorimetric Assay. Assay Range: 15.6 – 1,000 pg/ml. This standard curve is for demonstration purposes only. A standard curve must be generated for each assay.

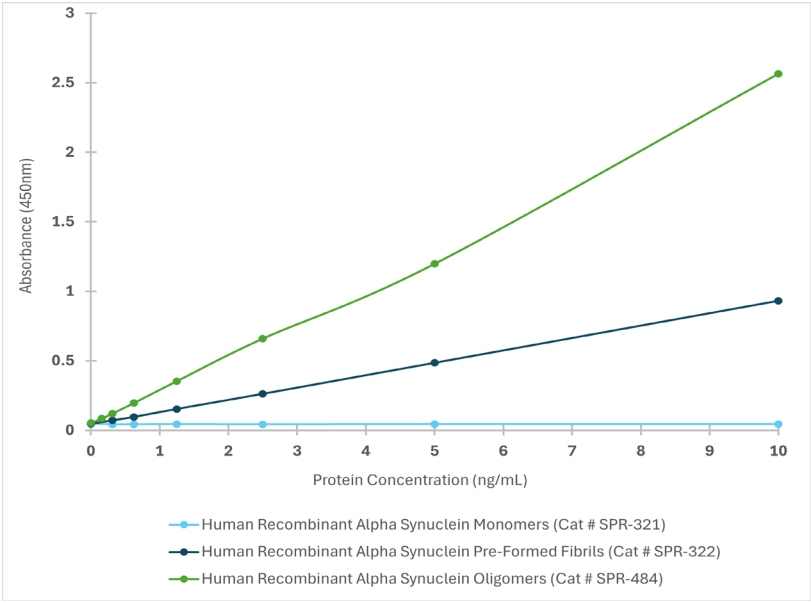

Figure 3. The graph demonstrates robust detection of Human Recombinant Alpha Synuclein Oligomers (Cat # SPR-484) and limited cross-reactivity with Human Recombinant Alpha Synuclein Pre-Formed Fibrils and Monomers (Cat #s SPR-322 & SPR-321).

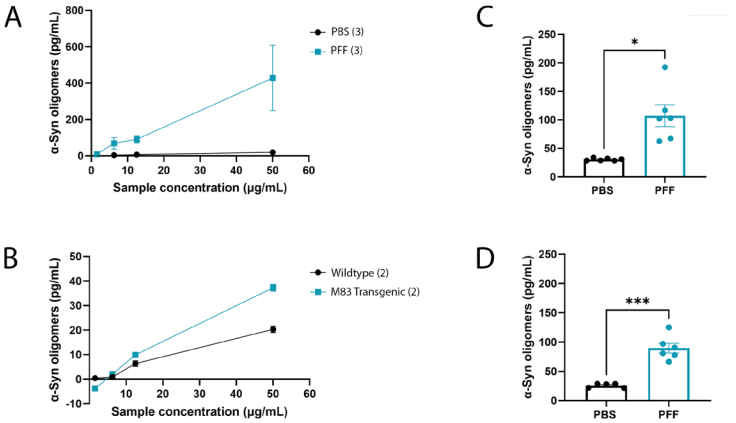

Figure 4. Representative quantification of mouse brain homogenate samples of unknown concentrations of Alpha Synuclein Oligomers against a standard curve of Human Recombinant Alpha Synuclein Oligomers when using StressMarq's Alpha Synuclein Oligomer ELISA kit (Cat # SKT-143). A: Non-detergent protein extraction from brain homogenates of hemizygous M83 mice injected in the striatum with either Human Recombinant Alpha Synuclein Pre-Formed Fibrils (PFF; n = 3) or with buffer (PBS; n = 3). B: Non-detergent protein extraction from brain homogenates of either homozygous M83 mice (M83 Transgenic; n = 2) or wildtype mice (Wildtype; n = 2). C-D: Detergent-based protein extraction (25 ug/mL) from brain homogenates of hemizygous M83 mice injected in the striatum with either PFF or PBS. Data from males (C: PFF n = 6; PBS n = 6) and females (D: PFF n = 6; PBS n = 5) are presented separately. Experimental data courtesy of Rodrigo Sandoval Contreras and Dareen Abdel-Rahman from the Prado Lab at University of Western Ontario, contributed as part of a collaborative research project.

Assay Range: 15.6 - 1000 pg/mL

- Suggested standard curve points are 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.65 pg/mL, and 0 ng/mL.

Assay Specificity and Species Reactivity

- This assay is specific for Alpha Synuclein Oligomer.

Sensitivity:

- The calculated sensitivity of the Alpha Synuclein Oligomer ELISA is 10 pg/mL.

Assay Limitations

- This assay has been validated for use with brain homogenates. Other sample types or matrices (e.g. urine, cerebrospinal fluid, cell culture supernatant, etc.) may contain interfering factors that can compromise the performance of the assay or produce inaccurate results.
- Some samples may contain higher levels of interfering factors that can produce abnormal results.
- If values for samples are not within the range of the standard curve, optimal sample dilutions need to be determined.
- The use of assay reagents not provided in this kit can compromise the performance of this assay.
- Do not mix components with reagents from other kits with different lot numbers.

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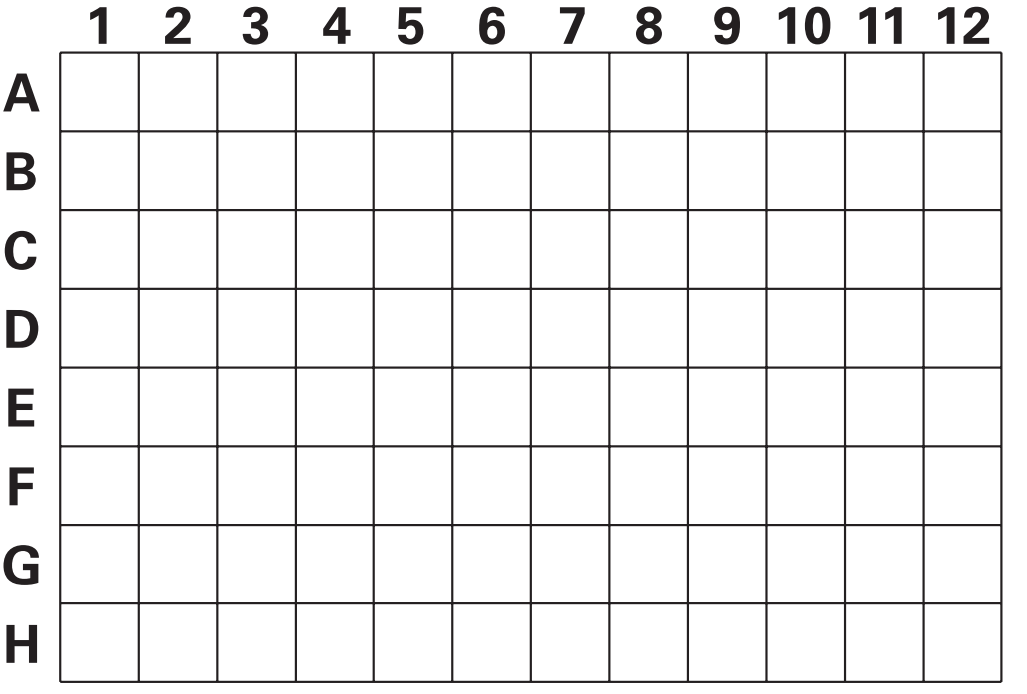
Warranty and Limitation of Remedy

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.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