STRESSMARQ
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Hsp70 and Hsp70/Hsc70

Immunohistochemistry Kit

Catalog# SKT-270

IHC GOLD™ SERIES

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Materials Supplied

Catalog Number	Item	Quantity/Size
SKC-240	Anti-Hsp70 Antibody (Special format of SMC-100, a mouse monoclonal. Does not cross react with Hsc70)	50µL
SKC-241	Anti-Hsp70/Hsc70 Antibody (Special format of SMC-106, a mouse monoclonal)	50µL
SKC-903	Amplifying IHC Wash Buffer (20x)	500 mL
SKC-905	Epitope Unmasking Solution (10x)	250mL
SKC-902	Amplifying Antibody Dilution Buffer	500mL
SKC-900	Antibody Amplifier™	1 (12 wells)
SKC-901	Antibody Amplifier Eclipse™ for IF	1 (12 wells)

If any of the items listed above are damaged or not in compliance wit the kit ordered, 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 IHC Gold™ 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

This kit will perform as specified if the antibodies are stored as directed at -20°C and the indicated buffers are stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied for ABC Protocol

1. Biotinylated Secondary Antibody
2. Vectastain Kit from Vector Labs .
3. DAB reagents
4. Xylene
5. Ethanol
6. 1x PBS

7. Peroxidase Blocking Solution (3% H_2O_2 in PBS)

   30% H_20_2.....2mL

   1X PBS.....18mL
8. Coverslips
9. Methyl Green (1% in water) or Hematoxylin (Gill-1)
10. Slide Racks and Holder
11. Permount
12. ddH_20
13. A standard laboratory rocker
14. Pipettors and tips
15. Mouse on Mouse Kit Vector Labs (This may need to be purchased if non-specific staining occurs when mouse antibodies are used on mouse tissues- separate protocol available on our website, www.stressmarq.com).

Materials Needed But Not Supplied for IF Protocol

1. Fluroescent-tagged Secondary antibody
2. Serum from secondary antibody's host
3. Xylene
4. Ethanol
5. Coverslips
6. Slide racks and holder
7. ddH_20
8. Prolong Gold Anti-Fade Solution (Invitrogen Catalog # S36936)

   OR

   Prolong Gold Anti-Fade Solution plus DAPI (Invitrogen Catalog # P36931)
9. A standard laboratory rocker
10. Pipettors and tips

INTRODUCTION

Background

Hsp70 genes encode abundant heat-inducible 70-kDa hsps (hsp70s). In most eukaryotes hsp70 genes exist as part of a multigene family. They are found in most cellular compartments of eukaryotes including nuclei, mitochondria, chloroplasts, the endoplasmic reticulum and the cytosol, as well as in bacteria. The genes show a high degree of conservation, having at least 5O% identity (1). The N-terminal two thirds of hsp70s are more conserved than the C-terminal third. Hsp70 binds ATP with high affinity and possesses a weak ATPase activity which can be stimulated by binding to unfolded proteins and synthetic peptides (2). When hsc70 (constitutively expressed) present in mammalian cells was truncated, ATP binding activity was found to reside in an N-terminal fragment of 44 kDa which lacked peptide binding capacity. Polypeptide binding ability therefore resided within the C-terminal half (3). The structure of this ATP binding domain displays multiple features of nucleotide binding proteins (4).

All hsp70s, regardless of location, bind proteins, particularly unfolded ones. The molecular chaperones of the hsp70 family recognize and bind to nascent polypeptide chains as well as partially folded intermediates of proteins preventing their aggregation and misfolding. The binding of ATP triggers a critical conformational change leading to the release of the bound substrate protein (5). The universal ability of hsp70s to undergo cycles of binding to and release from hydrophobic stretches of partially unfolded proteins determines their role in a great variety of vital intracellular functions such as protein synthesis, protein folding and oligomerization and protein transport.

About This Assay

This assay utilizes novel licensed technology aimed at removing limitations associated with the antibody staining of frozen and paraffin embedded tissue sections. The technology includes specific buffer formulations for antigen exposure, and optimized antibody formulation. It also includes a unique container called the Antibody Amplifier™. Incubating slides in antibody solution while rocking on a standard laboratory rocker guarantees even staining, and reproducible results. Please note that the antibody formulations used in this kit are specifically optimized for use in the IHC Gold™ Kit and may not work as a stand-alone reagent.

This assay has been designed to detect both the Hsp70/Hsc70 and Hsp70 content in frozen and paraffin-embedded sections.

Sample Types

Note: If cell lines or frozen sections are being used as opposed to paraffin-embedded ones, please use the following guidelines before moving onto the general protocols in the following section, performing the assay.

Preparation of Slides

A. Cell line:

1. Grow cultured cells on sterile glass cover slips.
2. Wash briefly with 1X PBS.
3. Fix 10 minutes in formalin (3.7% formaldehyde- dilute 37% formaldehyde 10X in 1X PBS). Keep wet.
4. Wash in 1X Amplifying Wash Buffer (Catalog# SKC-903) for 5 minutes.

B. Frozen Sections:

1. Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store frozen blocks at -80ºC.
2. Cut 4-8µm thick cryostat sections and mount on superfrost plus slides or gelatin coated slides. Store slides at -80ºC until needed.
3. Fix in formalin (3.7% formaldehyde- dilute 37% formaldehyde 10X in 1X PBS)
4. Wash in 1X Amplifying Wash Buffer (Catalog # SKC-903).

Once you have completed these, please move to Section 8 in the Assay Protocol on Page 9.

ASSAY PROTOCOL

1. Performing the Assay

Pipetting Hints

- Use different tips to pipette separate kit components
- 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.

IHC PROTOCOL USING THE ABC METHOD

1. Place slides in a glass slide holder and place in the following solutions serially (at room temperature):
   a. Xylene: 5 minutes swishing every 2 minutes (in fume hood).
   b. Xylene: 5 minutes, swishing every 2 minutes (in fume hood).
   c. 100% Ethanol: 2 minutes, swishing every 30 seconds.
   d. 95% Ethanol: 2 minutes, rocking
   e. 80% Ethanol, 2 minutes rocking.
   f. 70% Ethanol, 2 minutes rocking.
   g. 50% Ethanol, 2 minutes rocking.
   h. dd$H_2$0: 3 minutes, rocking. Repeat one time.
2. Wash with 1X Amplifying IHC Wash Buffer (Catalog # SKC-903) for 5 minutes, rocking at room temperature. Repeat once.
3. Wash with 1X Amplifying IHC Wash Buffer another 2 times, but for three minutes each this time.
4. Transfer slide holder to plastic, autoclavable container and submerge in 1X Epitope Unmasking Solution (Catalog # SKC-905).
5. Cover in foil and autoclave for 20-30 minutes.

6. Retrieve sample from autoclave and place on counter to let cool at room temperature for 20 minutes (Continuing emersion in Epitope Unmasking Solution (Catalog # SKC-905).
7. Wash with 1X Amplifying IHC Wash Buffer (Catalog # SKC-903) for 5 minutes, rocking at room temperature. Repeat once.

NOTE: For fatty tissue, such as brain or breast, use these directions for antigen retrieval instead of the auto-claving method above (Steps 4-7):

Place slides in slide rack. Lower slide rack into 600mL gently boiling 1X Epitope Unmasking Solution (Catalog # SKC-905). Boil gently for exactly 8 minutes, and then remove from the heat source (a large beaker with boiling chips on the bottom, sitting on a heat block works well). Slowly add 500 mLs of ddH_20 to bring buffer temperature down. Quickly put slide rack in glass slide holder and wash twice with 1X Amplifying IHC Wash Buffer (Catalog # SKC-903) for 5 minutes each times.

8. *Note: Use of the Antibody Amplifier™ is optional at this point. However, ensure that the slides are completely covered by the peroxidase blocking solution.*

   Wipe off back and sides of slide with a kimwipe, lay flat on bench top (in a humidity chamber if desired) and cover tissue with 1-3 drops of peroxidase block (3% H_2O_2 in PBS). Incubate at room temperature for 30 minutes, checking every 5-10 minutes to ensure the samples do NOT dry out (If they are starting to dry out, add more peroxidase block).
9. Put slides back into slide carrier and wash for 3 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903). Repeat two times.
10. Dilute primary antibody in Amplifying Antibody Dilution Buffer (Catalog # SKC-902). Use a minimum of 3mLs per slide for Antibody Amplifier. The suggested dilution for anti-Hsp70 and anti-Hsp70/Hsc70 is 1 in 10,000. Place lid onto the Antibody Amplifier™ and place on a standard laboratory rocker. Rock overnight at 4ºC. After use, store primary antibody at -20ºC. The antibody can generally be reused up to 4x without compromising sensitivity.
11. Put slides back into glass slide carrier and wash for 5 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903). Repeat once.
12. Incubate slides in biotinylated anti-mouse secondary antibody. Secondary is provided as part of the Vectastain kit by Vector Labs. Secondary antibody dilution is 1:2000

in Amplifying Antibody Dilution Buffer (Catalog # SKC-902), but optimal dilution is at the discrepancy of the end-user. Incubate slides rocking at room temperature for one hour in Antibody Amplifier*TM*. Remember that each slide needs 3mLs for full submersion.

13. As soon as the slides are incubating, mix up an ABC solution (found in Vectastain kit). The ABC solution is diluted 1:1000, but the optimal dilution is at the discretion of the user (the use of the Antibody Amplifier™ allows the user to significantly titrate out the ABC solutions). Remember if using the Antibody Amplifier*TM*, to make 3mLs per slide. Let the mixed solution sit at room temperature for 30-60 minutes before using.
14. Put slides in glass slide carrier and wash for 5 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903). Repeat once.
15. Incubate slides in pre-made ABC solution, rocking at room temperature for 1 hour in the Antibody Amplifier*TM*.
16. Make up DAB solution according to the manufacturer's instructions. 100-200µL per slide is needed. Incubate at room temperature for 20-30minutes.
17. Wash for 5 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903). Repeat once.
18. Wash for 3 minutes in ddH_20.
19. Wipe slides around tissue with kimwipes, keeping tissue moist, and add DAB solution. Check the staining of one slide first by testing a positive control: Incubate with 100-200µL of DAB solution, starting at 30 seconds. Monitor using the microscope at 40x. Keep note of DAB times. Wash slides as soon as staining is optimal with ddH_20.

    NOTE: All slides that you are comparing must be incubated for the same time period with DAB once this time is established with the positive control.
20. Wash slides with ddH_20 for 3 minutes.
21. Counter-stain using Methyl Green: Filter 3-10mLs of methyl green using a syringe filter into a 15mL tube. Add 200µL of methyl green onto tissue and incubate at room temperature for 2 minutes (or whatever time has been established). Rinse in containers of ddH_20 until slides are clean and let air dry, with slides standing vertically on their ends.
22. After tissues are completely dry, add one drop of Permount, put on coverslip, and smooth out bubbles using the big end of the pipettor tip. Label slides with: date, antibody, dilution and timing. After 4-5 hours, clean off extra Permount with Xylene

(The Xylene is optional- only if too much Permount was added, and it is covering tissue.)

IHC PROTOCOL USING KITS MANUFACTURED BY DAKO

For customers who prefer to use DAKO kit (DAKO Envision + System, Catalog # K4006, or DAKO Envision + System Catalog # K4011) please see our website www.stressmarq.com/technicalsupport for suggested protocols.

NOTE: The DAKO kits are highly recommended for mouse samples!

IHC PROTOCOL FOR DETECTING MOUSE TISSUES WITH MOUSE MONOCLONAL ANTIBODIES

For customers who are having problems with non-specific staining when mouse antibodies are used on mouse tissues, we recommend using the "Mouse on Mouse" kit from Vector Labs. Please see our website www.stressmarq.com for suggested protocols.

IMMUNOFLUORESCENCE PROTOCOL

The following outlines an IF protocol, using Alexa Fluor conjugated secondary antibodies on paraffin-embedded tissues, to allow optimal fluorescent detection markers for IHC.

1. Put slides in a glass slide holder and place in the following solutions serially at room temperature:
   a. Xylene: 5 minutes swishing every 2 minutes (in fumehood)
   b. Xylene: 5 minutes, swishing every 2 minutes (in fumehood)
   c. 100% Ethanol: 2 minutes, swishing every 30 seconds.
   d. 95% Ethanol: 2 minutes, rocking.
   e. 80% Ethanol: 2 minutes rocking.
   f. 70% Ethanol: 2 minutes rocking.
   g. 50% Ethanol: 2 minutes rocking.
   h. ddH_20: 3 minutes rocking. Repeat once.
2. Wash with 1x Amplifying IHC Wash Buffer (Catalog # SKC-903) for 5 minutes, rocking at room temperature. Repeat once.
3. Wash for 3 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903).

Repeat once.

4. Transfer slide holder to plastic, autoclavable container and immerse in 1X Epitope Unmasking Solution (Catalog # SKC-905).
5. Cover in tinfoil and autoclave for 20-30 minutes.
6. Retrieve sample from autoclave and place on counter to let cool at room temperature for 20 minutes (continue immersing in Epitope Unmasking Solution (Catalog # SKC-905).
7. Wash with 1X Amplifying IHC Wash Buffer (Catalog # SKC-903) for 5 minutes, rocking at room temperature. Repeat once.

NOTE: For fatty tissue, such as brain or breast, use these directions for antigen retrieval instead of the auto-claving method above (Steps 4-7):

Place slides in slide rack. Lower slide rack into 600mL of gently boiling 1X Epitope Unmasking Solution (Catalog # SKC-905). Boil gently for exactly 8 minutes, and then remove from the heat source (a large beaker with boiling chips on the bottom sitting on a heat block works well). Slowly add 500 mLs of ddH_20 to bring buffer temperature down. Quickly put slide rack in glass slide holder and wash twice with 1X Amplifying IHC Wash Buffer (Catalog # SKC-903) for 5 minutes each times.

8. Block for 30 minutes at room temperature using serum from secondary antibody's host.
9. Put slides back into slide carrier and wash in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903) for three minutes. Repeat twice.
10. Dilute primary antibody in Amplifying Antibody Dilution Buffer (Catalog # SKC-902). Use a minimum of 3mLs per slide for the Antibody Amplifier. The suggested dilution for anti-Hsp70 and anti-Hsp70/Hsc70 is 1 in 10,000. Place lid onto the Antibody Amplifier Eclipse™ and place on a standard laboratory rocker. Rock overnight at 4ºC. After use, store the antibody at -20ºC.
11. Put slides back into glass slide carrier and wash for 5 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903). Repeat once.
12. Prepare dilution of fluorescent anti-mouse secondary antibody. Secondary antibody needs to be diluted to 1:5000 in 1X PBS IN THE DARK. The optimal dilution is at the discretion of the end user (the use of the Antibody Amplifier™ allows the user to

significantly titrate our the antibody). If you wish to store and re-use the secondary antibody, make the dilution buffer as follows: 0.01M PBS pH 7.2, 0.05% thimerosal. This solution can be stored at 4ºC wrapped in tinfoil for 6 months, do not expose secondary antibody to light.

13. Incubate slides rocking at room temperature for 1-2 hours in the Antibody Amplified Eclipse™. Remember that each slide needs 3mLs.
14. Prepare Prolong Gold Anti-fade Solution. Take out of freezer and let thaw at room temperature for 1 hour. Do not artificially speed up warming.
15. Put slides in tinfoil wrapped glass slide carrier and wash for 5 minutes in 1X Amplifying IHC Wash Buffer (Catalog # SKC-903). Keep slides in the dark- do not expose to light. Repeat once.
16. Wash slides in ddH_20 for 3 minutes.
17. Prepare fluorescent microscope according to manufacturer's instructions.
18. Under dim lighting, use a kimwipe to dry most of the slide, keeping the mounted tissue moist. Add 1 drop of room temperature Prolong Gold Anti-Fade Solution to the center of tissue. Cover with a coverslip, gently removing air bubbles.
19. To view immediately, tack down the corners of the coverslip with clear nail polish. After viewing, to permanently seal the slide, paint the edges of the coverslip with the clear nail polish and let dry. Put the slides into the Antibody Amplifier Eclipse™, and allow the slides to cure in total darkness for 2-24 hours depending on the thickness of the tissue and the relative humidity of the environment.
20. To keep slides in optimal condition indefinitely, store them upright in a covered slide box containing a dessicant at -20ºC.

2. Notes on the Use of the Antibody Amplifier™

Key Notes:

1. Each Antibody Amplifier™ has 12 wells (1 slide per well), and therefore holds 12 slides.
2. Each well in the Antibody Amplifier™ requires approximately 3mLs of solution. Because of the characteristics of the amplification technology and chemistries used in this kit, it is important to use the correct amount of antibody. Please follow the instructions carefully for the relevant antibodies in each kit.

3. The Antibody Amplifier™ is designed to be stackable, and therefore, an experiment can be carried out with multiple Antibody Amplifiers™. We suggest, however, that you secure them together with laboratory tape.
4. Following placement of slides into the Antibody Amplifier™, the chamber with the lid on is placed on a standard laboratory rocker during incubation.
5. The Antibody Amplifier™ is chemical resistant (e.g. to xylenes).
6. The scientist can decide either to use the Antibody Amplifier™ during the entire procedure, or alternatively only during specific parts of the assay. In all cases, however, the scientist should use the Antibody Amplifier™ during antibody incubation.
7. Slides can be taken out of chambers with any pipette tip or forceps.
8. For more information, or trouble-shooting, please see our website (www.stressmarq.com), or call 250-294-9065.

General use of the Antibody Amplifier™ during a standard immunohistochemistry procedure using parrafin-embedded tissues:

As described in the above section, the scientist can decide either to use the Antibody Amplifier™ during the entire procedure, or alternatively only during specific parts of the assay. In all cases, however, the scientist should use the Antibody Amplifier™ during antibody incubation.

Note: When using brain tissue, it has become apparent that it comes off the slide quite easily. If it does, do not rock during the de-parraffinizing steps below. Rock at the lowest speed during antibody incubations. Also avoid pouring any liquids directly onto the tissue.

1. De-paraffinize slides (optional to use the Antibody Amplifier™). (If the scientist decides to use the Antibody Amplifier™ during this step, it is safe to do so, as the Antibody Amplifier™ is xylene and ethanol resistant).
2. Wash (optional to use the Antibody Amplifier™)
3. Antigen retrieval
4. Block (optional to use the Antibody Amplifier™)
5. Primary antibody (use the Antibody Amplifier™)

   Each well requires approximately 3 mLs to cover the slide.
6. Place the Antibody Amplifier™ on a standard rocker, place the lid on the chamber,

and incubate while rocking.

*If you choose to re-use the antibody, it can be stored at -20ºC, and in most cases can be re-used up to 4 times without loss of antibody efficiency.

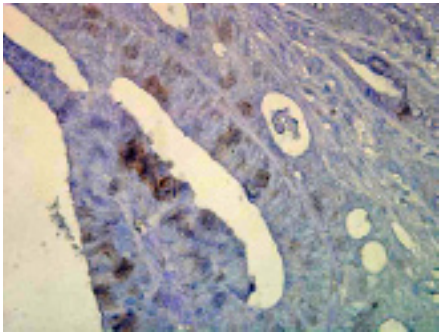
7. Wash (optional to use the Antibody Amplifier™).
8. Secondary antibody (use the Antibody Amplifier™)

   Note: The secondary antibody is usually tagged to allow for an amplification step.

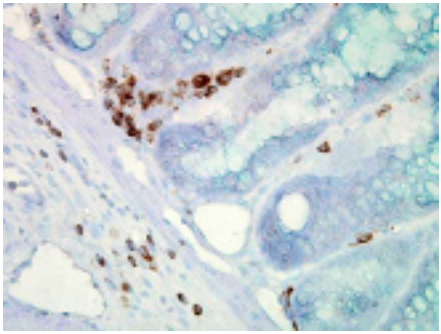
   Note: You can dilute the secondary antibody significantly more with the Antibody Amplifier™, the amount of dilution is dependent on the source of the secondary, but in our experience, secondaries can be diluted out 20 to 100 fold or more compared to the sedentary method.
9. Wash (optional to use the Antibody Amplifier™).
10. Amplifying step (e.g. avidin-biotin complex/HRP conjugate) (optional to use the Antibody Amplifier™).

    Note: You can dilute the amplifier significantly more with the antibody, the amount of dilution is dependent on the source and type of amplification.
11. Wash (optional to use the Antibody Amplifier™).
12. Substrate
13. Counter-stain

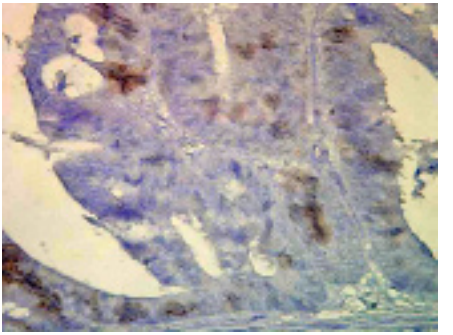
Validation Data



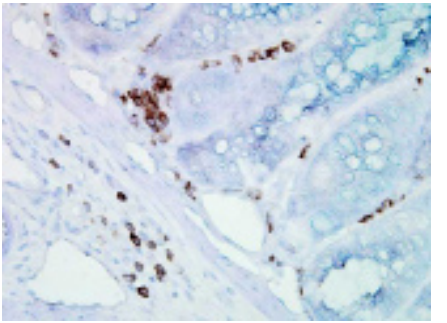
Anti-Hsp70. The human colon tissue stained (brown) mostly in inflammatory cells. Shown at a 1:10,000 dilution of SKC-240 using the ABC method.



Anti-Hsp70. The mouse colon tissue stained (brown) mostly in inflammatory cells. Shown at a 1:10,000 dilution of SKC-240 using the ABC method.



Anti-Hsp70/Hsc 70. The human colon tissue stained (brown) mostly in inflammatory cells. Shown at a 1:10,000 dilution of SKC-241 using the ABC method.



Anti-Hsp70/Hsc70. The mouse colon tissue stained (brown) mostly in inflammatory cells. Shown at a 1:10,000 dilution of SKC-241 using the ABC method.

Troubleshooting

Problem		Recommended Solutions
Weak or No Staining	Inadequate deparaffnization	Deparaffinize sections longer or change fresh xylene.
	Inactive primary antibodies- improper storage?	Replace with a new batch of antibodies. Aliquot antibodies into smaller volumes and avoid repearted freeze and thaw cycles.
	Antibody concentration was too low	Increase the concentration. Or run a serial dilution test to determine the optimal dilution that gives the best signal to noise ratio.
	Inadequate antibody incubation time	Increase antibody incubation time.
	Inadequate of improper tissue fixation	Increase duration of postfixation or try different fixatives..
	Tissue overfixation	Reduce the durationof postfix-ation. If the tissue has already been overfixed, perform the appropriate or recommended antigen retrieval procedure.
	Incompatible secondary and primary antibodies	Use secondary antibody that will intereact with the primary antibody.
	Inactive secondary antibody	Replace with a new batch of antibodies.

Problem		Recommended Solutions
	Inactive secondary antibody	Replace with a new batch of antibodies.
	Defective or incompatible enxyme substrate system	Replace with a new batch of reagents.
	Inadequate substrate incubation time	Increase the substrate incubation time.
	Incorrect mounting medium	Choose a correct mounting medium.
	Reagents applied in wrong order or steps omitted	Check notes or procedure used.
	It may simply be that there is no expression of the target protein!	
Overstaining	The concentration of primary and/or secondary antibodies was too high	Reduce antibody concentration or perform a titration to determine the optimal dilution for primary and secondary antibodies.
	Incubation time was too long	Reduce time.
	Incubation temperature was too high	Reduce temperature.
	Substrate incubation time was too long	Reduce substrate incubation time..
	Sections dried out	Avoid sections drying out, by adding the appropriate amount of solution.
High Background	Inadequate Washing	Wash at least three times between steps.

Problem		Recommended Solutions
High Background cont.	Diffusion of tissue antigen due to inadequate fixation	Increase duration of postfixation.
	Sections dried out	Avoid sections drying out by adding appropriate volumes of solution.
	Non-specific binding of primary antibodies to tissue	Non-specific binding may be reduced by using a higher dilution of primary antibodies.

References

1. Boorstein W. R., Ziegelhoffer T. & Craig E. A. (1993) *J. Mol. Evol.*38 (1): 1-17.
2. Rothma, J. (1989) *Cell* 59: 591 -601.
3. DeLuca-Flaherty *et al*. (1990) *Cell* 62: 875-887.
4. Bork P., Sander C. & Valencia A. (1992) *Proc. Nut1 Acad. Sci. USA* 89: 7290-7294.
5. Fink A.L. (1999) *Physiol. Rev.* 79: 425-449.

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.

NOTES

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