

MATERIAL SAFETY DATA SHEET

<p>1. IDENTIFICATION Product: See attached DATA SHEET Manufacturer: NeoMarkers Inc 47777 Warm Springs Blvd. Fremont, CA 94539 USA Tel 1-510-991-2800 Tel 1-800-828-1628 Fax 1-510-991-2826</p> <p>Authorized Representative: Lab Vision Products – Thermo Fisher Scientific 93-96 Chadwick Road, Astmoor Runcorn, Cheshire WA7 1PR, UK Tel: 44-1928-562600 Fax: 44-1928-562627 Labvision.uk@thermofisher.com</p>	<p>9. PHYSICAL AND CHEMICAL PROPERTIES Appearance: Clear liquid</p> <p>Odor: Odorless</p> <p>Solubility: Miscible with water</p> <p>pH: Neutral</p>
<p>2. COMPOSITION / INFORMATION ON INGREDIENTS Sodium Azide: NaN_3 MW: 65.01 Chemical Abstracts Registry Number 26628-22-8</p>	<p>10. STABILITY AND REACTIVITY Stability: Ab is stable for 24 months at storage conditions.</p> <p>Incompatibilities: Avoid contact with strong oxidizers. Dimethyl sulfate, acid chlorides, halogenated solvents, metals, acids, lead, and copper plumbing; forms highly explosive metal azides.</p>
<p>3. HAZARD IDENTIFICATION The material contains sodium azide as a preservative. Sodium azide is highly toxic. Direct contact of solution with skin may cause local dermatitis. Sodium azide in solution is readily absorbed through breaks in the skin and may cause systemic effects, the most common being hypotension and tachycardia. Sodium azide is a carcinogen. The National Institute of Occupational Safety and Health has issued a bulletin citing the potential explosive hazard due to the reaction of sodium azide with copper, lead, brass, or solder in plumbing systems. Sodium azide forms hydrazoic acid in acidic conditions and should be discarded in large volumes of running water to avoid formation of deposits in metal drainage pipes.</p>	<p>11. TOXICOLOGICAL INFORMATION To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been thoroughly investigated. Concentration in antibody solution: 0.09%. Parts per million (ppm) = 900 ppm. NIOSH Recommended Exposure Limit (REL) for skin exposure: Ceiling exposure level of 0.1ppm for a time-weighted average over a 10 hour workday (a ceiling exposure limit should not be exceeded at any time). Skin contact may cause dermatitis. Inhalation or ingestion or absorption through breaks in the skin can cause systemic effects, the most common being hypotension and tachycardia. Additional complaints include nausea, vomiting, diarrhea, headache, dizziness, temporary loss of vision, palpitation, dyspnea or temporary loss of consciousness or mental status decrease. (*) * Human and health effects of sodium azide exposure: A literature review and analysis. Chang, S and Lamm, SH, Int J Toxicol, 22:175-186, 2003.</p>
<p>4. FIRST AID MEASURES Eye Contact: Immediately flush with continuously running water.</p> <p>Skin Contact: Immediately wash the affected area with soap and water.</p> <p>Ingestion: Rinse mouth with water. Immediately seek medical help and call poison control center.</p> <p>Inhalation: Move to well-ventilated area and seek medical help if necessary.</p>	<p>12. ECOLOGICAL INFORMATION Data not yet available.</p> <p>13. DISPOSAL INFORMATION In the supplied form and concentration this product is not a hazardous material. Due to different local regulations, please check with a professional environmental consultant.</p>
<p>5. FIREFIGHTING MEASURES In the supplied form and concentration firefighting measures are Not Applicable. Packaging material may burn and release toxic fumes.</p>	<p>14. TRANSPORT INFORMATION In the supplied form and concentration this product is not a hazardous material. Please see product label to ensure proper transportation parameters such as temperature.</p>
<p>6. ACCIDENTAL RELEASE MEASURES Wear personal protective equipment, such as rubber gloves and boots, respirators, and goggles to prevent contact with self and others. Use paper towels or absorbent materials to absorb spills.</p>	<p>15. REGULATORY INFORMATION This product is not a hazardous material.</p> <p>16. OTHER INFORMATION No products are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties, or assurances as to the accuracy or completeness of information provided on our data sheets and website. Our warranty is limited to the actual price paid for the product. NeoMarkers is not liable for any property damage, personal injury, time or effort, or economic loss caused by our products.</p>
<p>7. HANDLING AND STORAGE Handling: See Section 8.</p> <p>Storage: Store vial at 2-8 °C in a dark environment</p>	
<p>8. EXPOSURE CONTROLS / PERSONAL PROTECTION Hand Protection: The use of gloves is required when handling this solution.</p> <p>Respiratory Protection: Liquid form does not require respirator.</p> <p>Ventilation: Liquid form does not require ventilation.</p> <p>Other Protective Equipment: Use lab coat to prevent contact with eyes, skin, and clothing, and face mask to prevent ingestion.</p>	



GENERAL PROTOCOL FOR IMMUNOHISTOCHEMISTRY

Formalin-fixed, paraffin-embedded tissues

I. SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

A. FIXATION

Place tissue in 4% paraformaldehyde or 10% buffered formalin for 6 to 12 hours.

B. DEHYDRATION

Dehydrate in alcohol gradient 80%, 95%, 100%, 100%, then clear in xylene.

C. EMBEDDING

Place fixed tissue in holder and dip holder into liquid paraffin until filled. Take out to cool until solidification.

D. SECTIONING / MOUNTING

1. Cut 5 μm of paraffin tissue and place tissue in hot water bath ($\sim 40^{\circ}\text{C}$) for 2-3 minutes.
2. Put on charged slides to bake at $60-70^{\circ}\text{C}$ for 2 hours.
3. Cool to room temperature for 2-3 hours and label slides according to conventional laboratory routine.

E. DEPARAFFINIZATION / REHYDRATION

1. De-wax slides in xylene (xylene substitute) for 3 x 5 minutes.
2. Hydrate slides in 100%, 100%, 95 %, 80% ethanol for 3 minutes each, then immerse slides in tap water for 5 minutes.
3. Put slides in Automation Buffer for at least 5 minutes, before starting the Staining Run. (It is recommended not to let slides dry).

II. IMMUNOHISTOCHEMISTRY PROTOCOL FOR LABVISION AUTOSTAINER

F. PRETREATMENT / ANTIGEN RETRIEVAL

If Heat Induced Epitope Retrieval (HIER) is recommended:

- a. Place slide rack in citrate, EDTA, or Tris HCl buffer (note SPECIFIC recommendations on data sheet regarding buffers).
- b. Heat samples to near boiling and keep at same temperature for 10-20 minutes. Some may require longer heating times and/or higher temperatures.
- c. Important: Cool slides in buffer at room temperature for at least 20 minutes before proceeding.
- d. Immerse in Automation Buffer at least 5 minutes before staining. (It is recommended to process Retrieved slides within 2 hours after heating, otherwise loss of unmasking effect might occur).

G. LOAD SLIDES ON AUTOSTAINER (Do not let slides dry)

H. PREPARE PROGRAM RUN OR RECALL FILE

I. ENDOGENOUS PEROXIDE QUENCHING (For horseradish peroxidase detection method)

In 3% hydrogen peroxide solution for 10 minutes, then wash slides in Automation Buffer.

J. PRIMARY ANTIBODY

1. Dilute primary antibody accordingly. For negative control, use PBS instead of primary antibody.
2. Incubate according to recommended time given on data sheet, then wash slides in Automation Buffer.

(The following instructions are specific to Lab Vision's UltraVision Detection Kit: TP-015-HD. For others, please follow manufacturer's recommendations).

K. SECONDARY ANTIBODY (LINK)

Incubate UltraVision biotinylated goat anti-polyvalent secondary antibody (if primary antibody is mouse monoclonal or rabbit polyclonal), for 10 minutes, then wash in Automation Buffer.

L. STREPTAVIDIN / PEROXIDASE (LABEL)

Incubate UltraVision streptavidin peroxidase for 10 minutes, then wash in Automation Buffer.

M. SUBSTRATE / CHROMOGEN

1. Mix DAB buffer and DAB chromogen according to UltraVision instructions (20 μL DAB chromogen per 1 mL DAB buffer).
2. Incubate for 5-10 minutes, wash slides in Automation Buffer. (Once DAB solution is prepared, it is stable for maximum 8 hours).

N. COUNTERSTAINING

1. Incubate in hematoxylin for 6-8 minutes (adjust accordingly) and wash in Distilled Water.
2. Remove slides from Autostainer.
3. Wash in running tap water 7-8 times, Automation Buffer for 3 minutes, then H_2O for 3 minutes.

III. MOUNTING

1. Put few drops of Permanent Mounting Media.
2. Cover with Glass Slide Coverslip.
3. Let mounted slides dry in oven for at least one hour.

