

Horseradish Peroxidase Labeled Lectin Staining Kit #1 (Cat. No.: HLK-001)

Kit Composition

The Horseradish Peroxidase Labeled Lectin Staining Kit #1 (HLK-series) contains 1mg each of the labeled lectins: Con A, DBA, SBA, WGA, UEA-I, PNA, GS-I, GS-II, BPA, MPA.

Lectin Specificity

Con A	α -D-Mannose, α -D-Glucose, Branched mannose.
DBA	Methyl-2-acetamido-2-deoxy-D-galactose.
SBA	α and β -N-Acetylgalactosamine > α and β -Galactose.
WGA	(GlcNAc- β -(1,4)-GlcNAc) ₁₋₄ > β -GlcNAc>Neu5Ac.
UEA-I	α -L-Fucose.
PNA	Terminal β -Galactose.
GS-I	Melibiose, α -D-Galactose.
GS-II	Terminal α - or β -N-Acetylglucosamine. The specific linkage of the N-Acetylglucosamine to the subterminal carbohydrate plays an important role in lectin binding
BPA	N-Acetylgalactosamine.
MPA	N-Acetylgalactosamine>Galactose.

Specific Applications

See individual datasheets for References.

Procedure For use

PRODUCT INFORMATION Horseradish Peroxidase Enzyme Activity Assay

Chemical Principle: Peroxidase + H₂O₂ → Complex
Complex + AH₂ (donor) → Peroxidase + H₂O + A (colored)

Assay Reagents: BUFFER: 0.01M Sodium phosphate, pH 6.0.

ENZYME: Dilute with Buffer. Acceptable dilution: 1-2 μ g/ml.

DYE: 1% o-dianisidine in methanol prepared fresh daily. Store in amber bottle or wrapped in foil.

SUBSTRATE: Prepare 0.3% H₂O₂ solution in deionized or distilled water from stock H₂O₂ solution Prior to use dilute to a final concentration 0.003% in Buffer.

Procedure:

1. Add 0.05 ml of DYE to 6.0 ml of SUBSTRATE. Add 2.9 ml to Reaction test tube and 2.9 ml to Control test tube.
2. At time=0, add 100 μ l of diluted ENZYME to Reaction tube and 100 μ l PBS to Control tube. Mix thoroughly.
3. Measure and record optical density at 460nm (OD460) every 15 seconds for 3 minutes, or take the end point reading after 3 minutes by stopping the reaction with 100 μ l of concentrated NaN₃.
4. Use this value to determine the rate of change in absorbance per minute.

Enzyme Activity Calculations:

One unit of peroxidase activity is that amount of enzyme decomposing 1 μ mole of peroxide/minute at 25°C. 11.3 x 10³ cm⁻¹ is the molar absorbance of H₂O₂.

$$\text{OD460/ min} = \frac{\text{OD460/ 3min} - \text{OD Control / 3minutes}}{3\text{minutes}}$$
$$\text{mg enzyme/ ml reaction mixture} = \frac{[\text{enzyme dilution}]}{30}$$
$$\text{units / mg} = \frac{\text{OD460/ min}}{11.3 \times \text{mg enzyme / ml reaction mixture}}$$

Caution: Due to inhibitory sugar present in the conjugates solution, to dilute the Conjugate 50-100 times with buffer before assay.

Additional Products

In addition to more than 300 labeled lectins, EY Laboratories, Inc. also manufactures a large selection of carbohydrate gels for lectin purification, antibody gels for purification of primary antibodies, and a number of different protein/glycoprotein gels. For further information, please contact customer service at EY Laboratories, Inc.

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Horseradish Peroxidase Labeled Lectin Kit #1 Product Information

Catalog Number: H-1104-1

Description: Pure *Canavalia ensiformis* lectin (Con A) from Jackbean, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg/1ml purified Con A Horseradish Peroxidase.
(Based on OD280)

Carbohydrate Specificity: α -D-Mannose, α -D-Glucose, Branched mannose.

Inhibitory Carbohydrate: Methyl α -D-Mannopyranoside >> α -D-Mannose>> α -D-Glucose.

Activity: Con A is a relatively weak blood agglutinin. More than 10 μ g/ml may be required to give visible agglutination of neuraminidase treated human erythrocytes.

Buffer: 0.05 M Tris - 0.15M NaCl-0.004M CaCl₂, pH 7.0-7.2.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References :

1. Hori, T., et al. (1985). Acta Neuropath. (Berlin). **66** : 177.
2. Ree, H.J. (1983). Cancer. **51** : 1639-1646.

Catalog Number: H-1201-1

Description: Pure *Dolichos biflorus* lectin (DBA) from horsegram, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified DBA Horseradish Peroxidase / 1 ml Buffer.
(Based on OD280)

Carbohydrate Specificity: Methyl-2-acetamido-2-deoxy-D-galactose.

Inhibitory Carbohydrate: Terminal α -D-Acetylgalactosamine.

Activity: 4 μ g/ml will agglutinate human type A₁ cells. As much as 200 μ g/ml is needed to agglutinate type A₂ cells.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Procedure for Use: Dilute with 1% BSA PBS at least 100 x before use

References:

1. Etzler, M.E. and Kabat, E.A. (1970). Biochemistry. **9** : 869-877.

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Horseradish Peroxidase Labeled Lectin Kit #1 Product Information

Catalog Number: H-1301-1

Description: Pure *Glycine max* lectin (SBA) from soybean, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg affinity purified SBA Horseradish Peroxidase / 1 ml Buffer.
(Based on OD280)

Carbohydrate Specificity: α and β - N-Acetylgalactosamine > α and β -Galactose.

Inhibitory Carbohydrate: Terminal α - and β - N-Acetylgalactosamine>Galactose.

Activity: Less than 4 μ g/ml will agglutinate fresh A₁ cells. Older B cells can react stronger than A₂ cells.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze-thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Lis, H. and Sharon, N., (1973) Ann.Rev. of Biochem., **42** : 541-574.
2. Den, H., et al., (1975) J.Cell.Biol. **67** : 826-834.
- Hammerstrom, et al., (1977) Biochem.

Catalog Number: H-2101-1

Description: Pure *Triticum vulgare* lectin (WGA) from wheat germ, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified WGA Horseradish Peroxidase / vial.
(Based on OD 280)

Carbohydrate Specificity: (GlcNAc- β -(1,4)-GlcNAc)₁₋₄> β -GlcNAc>Neu5Ac

Inhibitory Carbohydrate: GlcNAc β (1,4) GlcNAc β (1,4) GlcNAc>GlcNAc β (1,4) GlcNAc>GlcNAc>>sialic acid(Neu5Ac)>>GalNAc

Activity: Less than 4 μ g/ml will agglutinate human type O erythrocytes. Less than 1 μ g/ml will agglutinate neuraminidase treated erythrocytes.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store lyophilized powder refrigerated at 5 - 8°C or frozen. Store liquid frozen in aliquots. Avoid freeze-thaw cycles.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Peters, B.P., et al. (1979) Biochemistry. **18** : 5505-5511.
2. Lotan, R. et al. (1975) Biochem. Biophys. Res. Comm. **62** : 144-150.
3. Ebisu, S., et al. (1977) Carbohydrate Res. **58** : 187-191.
4. Watanabe, K. and Hakomori, S. -I. (1973) FEBS Lett. **37** : 317-320.
5. Yamamoto, K., et al. (1981) Biochemistry. **20** : 5894-5899.

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Horseradish Peroxidase Labeled Lectin Kit #1 Product Information

Catalog Number: H-2201-1

Description: Pure *Ulex europaeus* lectin (UEA-I) from gorse, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified UEA-I Horseradish Peroxidase / 1ml Buffer.
(Based on OD280)

Carbohydrate Specificity: α -L-Fucose.

Inhibitory Carbohydrate: α -L-Fucose.

Activity: Less than 4 μ g/ml will agglutinate human type O erythrocytes. Less than 0.5 μ g/ml will agglutinate neuraminidase treated erythrocytes.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Holthofer, H. et al. (1982) Lab. Investigation. **47** : 60-66.
2. Miettinen, M., et al. (1983) Am. J. Clin. Path. **79** : 32.
3. Walker, R.A. (1985) J. Pathology. **146** : 123-127.
4. Allen, J.U. and Bosslet, K. (1988) Am. J. Clin. Path. **90** : 463-471.
5. Oriol, R., et al. (1986) Vox Sang. **51** : 161-171.
6. Torrado, J. et al. (1989) Am. J. Clin. Path. **91** : 503 (Letter to the Editor).

Catalog Number: H-2301-1

Description: Pure *Arachis hypogaea* lectin (PNA) from peanut, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified PNA Horseradish Peroxidase / 1 ml Buffer.
(Based on OD 280)

Carbohydrate Specificity: Terminal β -Galactose.

Inhibitory Carbohydrate: Lactose > β -Galactose.

Activity: Less than 1 μ g/ml will agglutinate human erythrocytes neuraminidase treatment of the cells.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze-thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Cooper, H.S. (1984). Human Pathology. **15** :904-906.
2. Moller, P. (1982) Virchows Arch. **396**: 313-317.
3. Vierbuchen, M. and Klein, P.J. (1983). Laboratory Inv. **48** (2) : 181.
4. Ree, H.J. and Hsy, Su-ming. (1983). Cancer. **51** : 1631.

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Horseradish Peroxidase Labeled Lectin Kit #1 Product Information

Catalog Number: H-2401-1

Description: Pure *Griffonia simplicifolia* lectin (GS-I), Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1mg purified GS-I Horseradish Peroxidase.
(Based on OD280)

Carbohydrate Specificity: Melibiose, α -D-Galactose.

Inhibitory Carbohydrate: α -Galactose.

Activity: 20-30 μ g/ml is required to agglutinate fresh type B blood cells. Lectin activity against all blood types increases after neuraminidase treatment of the cells.

Buffer: 0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl₂, pH 7.2 - 7.4

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Remarks: Calcium is REQUIRED for binding. 0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

References:

1. Murphy, L. A. and Goldstein, I. J. (1977). J. Biol. Chem. 252 : 4739-4742.
2. Judd, W. J., et al. (1978). Transfusion (Philadelphia). 18 : 274-280.
3. Eckhardt, A. E., et al. (1982). Cancer Res. 42 : 2977-2979.
4. Maddox, D. E., et al. (1982). PNAS. 79 : 166-170.

Catalog Number: H-2402-1

Description: Pure *Griffonia simplicifolia* lectin (GS-II), Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified GS-II Horseradish Peroxidase / 1 ml Buffer.
(Based on OD280)

Carbohydrate Specificity: Terminal α - or β - N-Acetylglucosamine. The specific linkage of the N-Acetylglucosamine to the subterminal carbohydrate plays an important role in lectin binding.

Inhibitory Carbohydrate: N-Acetylglucosamine.

Activity: 5-10 μ g/ml will agglutinate T_k polyagglutinable cells.

Buffer: 0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl₂, pH 7.2 - 7.4

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Remarks: Calcium is REQUIRED for binding. 0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

References:

1. Murphy, L. A. and Goldstein, I. J. (1977). J. Biol. Chem. 252 : 4739-4742.
2. Shanker Iyer, P. N., et al. (1976). Arch. Biochem. Biophys. 177 : 330-333.

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Horseradish Peroxidase Labeled Lectin Kit #1 Product Information

Catalog Number: H-2501-1

Description: Pure *Bauhinia purpurea* lectin (BPA) from Camel's foot tree, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified BPA Horseradish Peroxidase / 1ml Buffer.
(Based on OD280)

Carbohydrate Specificity: N-Acetylgalactosamine.

Inhibitory Carbohydrate: N-Acetylgalactosamine.

Activity: Less than 0.5 µg/ml will agglutinate human erythrocytes after neuraminidase treatment of the cells. Without prior enzyme treatment, at least 25 µg/ml is required to agglutinate red blood cells.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Irimura, T. and Osawa, T.(1972).Arch. Biochem. Biophys. **151** : 475-482.
2. Imai, Y. and Osawa, T.(1983).Scand.J.Immunol. **18** : 217-224.

Catalog Number: H-3901-1

Description: Pure *Maclura pomifera* lectin (MPA) from Osage Orange, Horseradish Peroxidase conjugated.

Lot Number:

Protein Concentration: 1 mg purified MPA Horseradish Peroxidase / 1ml Buffer.
(Based on OD280)

Carbohydrate Specificity: N-Acetylgalactosamine>Galactose.

Inhibitory Carbohydrate: Melibiose [Gal α(1,6) Glc]>α-D-Galactose.

Activity: Less than 5 µg/ml will agglutinate type O human erythrocytes. Less than 0.1 µg/ml will agglutinate neuraminidase treated cells.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Allen, P.Z. (1985) Infect. Immunol. **47** : 90-93.
2. Chuba, J.V. and Kuhns, W. (1973) Nature (London) **242** : 342.
3. Jones, J.M. and Feldman, J.D. (1973) J. Immunol. **111** : 1765.

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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006

Revision 4

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MSDS for Horseradish or Alkaline Phosphatase Labeled Proteins & Biotin Continued - page 2 of 2.

PRODUCT IDENTIFICATION

Name: Purified proteins or biotin labeled with Horseradish Peroxidase or Alkaline Phosphatase.
Catalog Number (s): HP-02, BA-104, BA-105, BA-108, BA-109, H-1102 to H-9000, LA-1104 to LA-9000, PA-2100 to PA-2701, AA-2100 to AA-2701, HAF-001 to HAF-2354, AAF-001 to AAF-2354, HA-01 to HA-013, AA-01 to AA-013, HAL-1104 to HAL-4701, AAL-1104 to AAL-4701.
Synonyms: Protein A, Avidin (egg white), Biotin, Lectins, Secondary Antibodies labeled with Horseradish Peroxidase or Alkaline Phosphatase.

EMERGENCY INFORMATION

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EMERGENCY PHONE:

650 342 3296

HAZARDOUS COMPONENTS

Specific protein(s) as listed on the vial label. Solutions are at a concentration generally greater than 0.5mg protein/ ml. Biological activity of these labeled proteins will vary. Horseradish Peroxidase and Alkaline Phosphatase are both potent enzymes which may be harmful if ingested, inhaled, or allowed to absorb through the skin. Both enzymes are known to cause allergic responses in sensitive individuals.

HEALTH HAZARD INFORMATION

EXPOSURE LIMITS: None established. The toxicological properties of these products have not been thoroughly investigated. Care should be taken when handling any of these materials.
EFFECTS OF OVEREXPOSURE: May causes localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure.
ROUTES OF EXPOSURE: Inhalation of powders and skin contact with liquids are the primary routes of exposure. Care should be taken to avoid the formation of aerosols when handling any of the solutions.

PHYSICAL CHARACTERISTICS

APPEARANCE: Powders are a light brown. Solutions will be light to dark brown.
SOLUBILITY: Powders are completely soluble in many biological buffers and water. All liquids are completely miscible in water and biological buffers.

FIRE AND EXPLOSION HAZARDS

EXTINGUISHING MEDIA: Not considered to be a fire hazard.
SPECIAL FIRE FIGHTING PRECAUTIONS: Water spray or CO₂.
None required.

NOTE:

Alkaline Phosphatase conjugates contain less than 0.05% sodium azide as a preservative. Azide may react with lead and copper plumbing to form explosive metal azides. Flush with copious amounts of water when disposing material in the sink.

REACTIVITY DATA

STABILITY: Stable. The nature of any decomposition products are not known. They are not believed to be hazardous.
HAZARDOUS POLYMERIZATION: Will NOT occur.
INCOMPATIBILITY: None known. (Lead and copper may react with sodium azide).

SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL: Avoid contact with powder or liquid. Clean up spill with a paper towel soaked in household bleach. Do not allow solutions to dry on environmental surfaces. Wash affected area with detergent after the area has been treated with bleach.
WASTE DISPOSAL: Incinerate, autoclave, or dispose of paper waste in accordance with all Local, State, and Federal regulations. Due to the small quantities of material involved these products are generally not considered to be environmental hazards. All of these proteins are fully biodegradable.

EMERGENCY FIRST AID PROCEDURES

May be harmful if swallowed, inhaled, or allowed to absorb through the skin. Wash contacted area with water for 15 minutes. If inhaled remove to fresh air. Report exposure to the appropriate safety official. Consult a physician if irritation occurs or if there is any indication of an allergic response, such as watering eyes, sneezing, or difficulty breathing. Any eye contact should be reported to a physician immediately

SPECIAL HANDLING PRECAUTIONS

VENTILATION: No special ventilation is required but it is recommended to handle these reagents in a fume hood when possible.
EYE PROTECTION: Required. Goggles or safety glasses with a side shield are recommended.
RESPIRATORY PROTECTION: Recommended as a safety precaution, specifically when working with powders. An approved respirator may be required for those individuals already known to be sensitive to these materials.
PROTECTIVE GLOVES: Required when handling any of these materials.

SPECIAL PRECAUTIONS

This material is for research and experimental application only. It is not intended for food, drug, household, agricultural, or cosmetic use. All materials should be handled only by technically qualified individuals experienced with working with potentially hazardous chemicals. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information, based on all sources available. EY Laboratories, Inc. shall not be held liable for any damage resulting from handling or contact with the above product.

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