Kit #1 One Minute Kit for Carbohydrate Identification

Catalog No. IC-LG-001-5

Kit Contents

10 Units (2x5 strips) Pre-Innoculated Con A InstantChek Device
10 Units (2x5 strips) Pre-Innoculated WGA InstantChek Device
10 Units (2x5 strips) Pre-Innoculated ECA InstantChek Device
10 Units (2x5 strips) Pre-Innoculated UEA-I InstantChek Device
10 Units (2x5 strips) Pre-Innoculated DBA InstantChek Device

10 Units (2x5 strips) Blank InstantChek Device

Reagents:
3 x 5 Different lectin-gold conjugates in lyophilized form.
(Con A, WGA, UEA-I, ECA, DBA)
1x 5ml Prewet Solution
1x 10ml Reconstitution Buffer
1x 10ml Wash Buffer
1x 1 InstantChek™ Lectin-Gold test kit insert

If you have any questions, please contact us directly at:

www.eylabs.com
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Introduction

The InstantChek™ Carbohydrate I cation test kit is a simple, rapid sensitive specific test for rapid testin rbohydrates that bind to lectins.

Lectin-Gold	Carbohydrat cificity
Con A	Terminal Mannose or Branched Mannose
WGA	GlcNAc or Sialic Acid
ECA	Gal A or B – linked
UEA-I	L-Fucose alpha linked
DBA	GalNAc alpha-linked

The lectin-gold conjugate is red in color and acts as a signal generator when bound to a specific carbohydrate of a glycoprotein or oligosaccharide.

Chart 1. Volume of lectin-gold to use for reconstitution

Lyophilized Lectin-Gold	Gold Diluent (GD) for reconstitution
Con A	400ul
WGA	500ul
ECA	200ul
UEA I	400ul
DBA	400ul

CAUTION: Store above reagents in above 4º Celsius in refrigerator. AVOID FREEZING

Upon standing at room temperature or refrigeration temperature, the conjugates of the lectin gold may aggregate. Remove aggregate before use by lab bench top centrifuge at 5000 rpm for 5 minutes. Failure to do so will generate false positive results. Discard ppt and use supernatant for experiment.

Applications of reconstituted lectin gold include

a. Use with this InstantChek carbohydrate identification kit
b. For Light Microscopy studies.

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Indirect Method – to detect a Carbohydrate of a Glycoprotein

A sandwich assay can be made of many different forms. For example, two of the same lectin can bind to a glycoprotein. Two different lectins may also bind to the same glycoprotein.

Assay Preparation
To begin, select an InstantChek™ device pre-inoculated with lectin that is specific for the sugar you are detecting. For example, the Con A device is specific for Terminal Mannose or Branched Mannose.

Preparation of Reagents
Reconstitution of Lyophilized Lectin-Gold – See Chart 1.

Indirect Method Assay Protocol
This protocol may be used for all five lectin-gold reagents.

1. Add 20µl Prewet solution. Let it absorb completely.
2. Add 40µl Glycoprotein (1mg/ml). Let it absorb completely.
3. Add 20µl Reconstituted Lectin Gold that is specific for your carbohydrate. Let it absorb completely.
4. Wash by adding 80µl Wash solution Twice (2 x 80µl) . Read Results.

N.B. For best results in testing fucose-linked glycoproteins, add after the 3rd step, 1 drop gold diluent before the wash step. This enables the complex to become more stable.

Interpretation of Results
- Red to Pink dot indicates a Positive Result
- No dot indicates a Negative Result.

NOTE: This kit does not work on a glycoprotein's carbohydrate moiety that is facing the membrane. This is because the sugar is sterically hindered from binding with the lectin.

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Direct Method – to ect a Carbohydrate of a Glycoprotein

Preparation of Reagents
Reconstitution of Lyophilized Lectin – See Chart 1.

Assay Preparation
1. Select a blank device.
2. Inoculate 1-3 µg/ml of a glycoprotein at the center of the device membrane and let it air dry over one hour, or overnight. Once dry the device is now ready for the Assay Protocol.

Direct Method Assay Protocol
1. Add 10µl Prewet solution to center of device. Let it absorb completely.
2. Add 20µl of the reconstituted Lectin-Gold that is specific for your carbohydrate. Let it absorb completely.
3. Add 50µl Wash Buffer. Read Results.

Interpretation of Results
- Red to Pink dot indicates a Positive Result
- No dot indicates a Negative Result.

NOTE: This kit does not work on a glycoprotein's carbohydrate moiety that is facing the membrane. This is because the sugar is sterically hindered from binding with the lectin.

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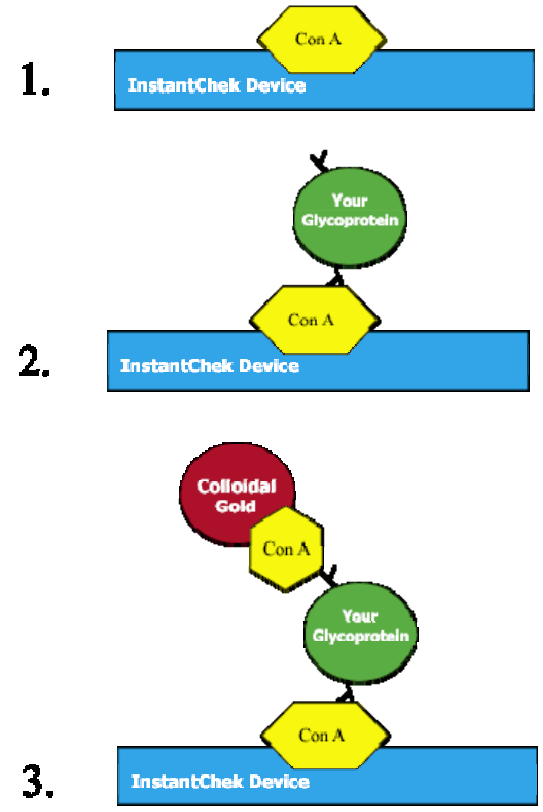
Indirect Method - Sandwich Assay



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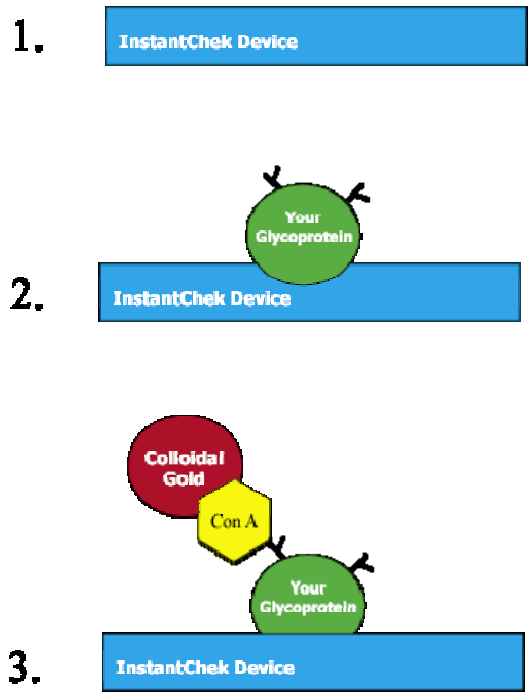
Direct Method



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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 5

PRODUCT IDENTIFICATION

Name:	InstantChek Carbohydrate Identification Kit
Catalog Number(s):	IC-LG-001-5, IC-LG-002-5, IC-LG-003-5
Synonyms:	5 Lectin-Gold Indirect and Direct Assay kit.
Formula:	N/A

EMERGENCY INFORMATION

EY Laboratories, Inc.
107 North Amphlett Blvd.
San Mateo, CA. 94401

EMERGENCY PHONE**: 650 342-3296**

HAZARDOUS COMPONENTS

Specific lectin listed on the vial label. They are colloidal gold labeled and lyophilized. Buffer is in Phosphate or Tris buffer saline (pH 7.4-8).

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 cause localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure. The known effects are due to the protein.
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 ruby red. Solutions will be clear to dark brown or red.
SOLUBILITY:	Powders are completely soluble in many biological buffers and water.

FIRE AND EXPLOSION HAZARDS

Not considered to be a fire hazard

EXTINGUISING MEDIA:	Water spray or CO_2.
SPECIAL FIRE FIGHTING NOTE:	None required.
NOTE:	Most solutions 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.

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REACTIVITY DATA

STABILITY:	Stal :omposition products are not known to be h us.
HAZARDOUS POLYMERIZATION:	Will :cur.
INCOMPATIBILITY:	Nor /n. (Lead and copper may react with sod de).

SPILL/LEAK PROCEDURES

MATERIAL RELEASE/SPILL:	Avoic :t with powder or liquid. Clean up spill with a 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.

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:	Not required under most circumstances but recommended as a safety precaution.
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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