INSTRUCTIONS FOR USE

INDOLE (DMACA) REAGENT – p-Dimethylaminocinnamaldehyde

PRODUCTS

AS-701 Indole (DMACA) Reagent – p-Dimethylaminocinnamaldehyde 1 bottle / pkg

INTENDED PURPOSE

Spot biochemical reagents are intended for in vitro diagnostic use by trained laboratory personnel for the identification and differentiation of microorganisms based on specific biochemical characteristic, such as enzymatic activity or metabolic reactions.

INTENDED USERS

Scientists, laboratory, and healthcare professionals trained in anaerobic microbiology techniques working in areas such as clinical, research, industrial, pharmaceutical and veterinary applications.

FORMULATION*

Indole Reagent is designed for use in the Spot-Indole Test to detect indole production by anaerobic bacteria. It determines an organism's ability to produce indole from the deamination of tryptophan by the enzyme tryptophanase. This reagent has been shown to be 10 to 100 times more sensitive than Kovac's or Ehrlich's reagents in detecting indole and its derivatives. Indole reacts with p-Dimethylaminocinnamaldehyde (DMACA) to produce a blue to blue-green compound, indicating a positive result.

p-Dimethylaminocinnamaldehyde	10.00	g
Hydrochloric acid	100.00	mL
DI Water	890.00	mL

*Approximate formula. Adjusted and/or supplemented as required to meet performance criteria.

Final volume: 30.0 mL ± 3.0 mL

PRECAUTIONS

For *IN VITRO DIAGNOSTIC USE* only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use by properly trained and qualified personnel only. Sterilize all biohazard waste prior to disposal.

Avoid contact with skin, as it may cause irritation. In case of skin contact, immediately remove contaminated clothing and rinse the affected area thoroughly with water. In case of eye contact, rinse cautiously with water for several minutes. Always wear appropriate personal protective equipment (PPE), including gloves, lab coat, and eye protection, when handling reagents.

Report serious incidents that occur in direct relation to this product to tech@biolog.com. As necessary, report serious incidents to the regulatory authority in which the user is established.

When working with anaerobic culture media, the potential for ergonomic hazards may exist due to repetitive motions, awkward postures, improper bench/chair heights or poor lighting. Although it is beyond the scope and provision of products by Anaerobe Systems, it should be recognized and mitigated by the end user in the laboratory environment.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at room temperature (15 – 25°C) until used. Avoid overheating or freezing. Avoid any unnecessary exposure to light. Do not use the product if there are signs of deterioration to the package. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the label.

Shelf Life: 1 year from the date of manufacture.

PROCEDURE

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transportation of anaerobes. The selection of specimens for culture is made by physicians or scientists collecting the sample.

Spot-Indole Test: Inoculate an ager medium containing sufficient tryptophan, such as Egg Yolk Agar (EYA; catalog #: AS-511) or Brucella Blood Agar (BRU; catalog #: AS-111). Place a sterile filter paper disk on an area of heavy growth for 5 minutes. Remove the disk to an empty petri dish and add one drop of Indole Reagent to the disk. The development of a blue or blue-green color on the filter disk within 30 seconds indicates a positive result for indole production. Dark-pigmented organisms should be examined carefully, as pigmentation may mask the color change.

Alternatively, moisten a piece of filter paper in a clean petri dish with Indole Reagent, taking care not to oversaturate the paper. Remove several colonies from the agar with a loop or wooden applicator and rub them into the moistened filter paper. Using a heavy inoculum is preferred when testing anaerobes since the reaction can sometimes be weak.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

INTERPRETATION OF RESULTS

Development of a blue or blue-green color on the filter disk or around the inoculum within 30 seconds indicates a positive result for indole production. Dark-pigmented organisms should be examined carefully, as pigmentation may mask the color change. No color change or development of a pink color within 30 seconds is considered negative for indole production. Any color change occurring after 30 seconds should be disregarded.

LIMITATIONS

A growth medium containing tryptophan is essential when testing for indole production. Because the enzyme that degrades tryptophan is diffusible in agar, only one culture should be tested per plate to avoid false-positive results. Do not use plates containing a nitrate disk, as a positive nitrate reaction may interfere with the Spot-Indole Test and cause false-negative results. Indole Reagent alone does not provide complete information for bacterial identification. Additional tests and media are necessary for full identification. Consult reference materials for further details.

QUALITY CONTROL

The following organisms are routinely used for quality control testing at Anaerobe Systems.

Organism Tested	ATCC® #	Expected Results
Bacteroides fragilis	25285	Negative
Peptostreptococcus anaerobius	27337	Negative
Fusobacterium necrophorum	25286	Positive
Cutibacterium acnes (indole+ strains)	6919	Positive

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

To evaluate the reactivity and performance of this reagent, it is recommended to test the following ATCC® strains grown on AS-111 Brucella Blood Agar plates.

Organism	ATCC® #	Expected Results
Bacteroides fragilis	25285	Negative
Fusobacterium necrophorum	25286	Positive

Physical Appearance: Indole Reagent should appear as a clear amber liquid within an opaque plastic bottle.

ATCC® is a registered trademark of American Type Culture Collection.

REFERENCES

1. MacFaddin JF. *Biochemical Tests for Identification of Medical Bacteria.* 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.

2. Leber AL, Burnham CA, eds. *Clinical Microbiology Procedures Handbook*. 5th ed. 4 vols. Washington, DC: ASM Press; 2023.

3. Carroll KC, Pfaller MA, eds. *Manual of Clinical Microbiology*. 13th ed. 4 vols. Hoboken, NJ: Wiley-Blackwell; 2023.

4. Jousimies-Somer HR, Sutter VL, eds. *Wadsworth-KTL Anaerobic Bacteriology Manual*. 6th ed. Belmont, CA: Star Publishing Company; 2002.

5. Sutter VL, Cater WT. Evaluation of media and reagents in indole-spot test in anaerobic bacteriology. *Am J Clin Pathol*. 1972;58(3):335–338.

6. Lombard GL, Dowell VR Jr. Comparison of three reagents for detecting indole production by anaerobic bacteria in microtest systems. *J Clin Microbiol*. 1983;18(3):609–613.

GLOSSARY OF SYMBOLS

SYMBOL	TITLE	DESCRIPTION	STANDARD	REF#
REF	Catalog number	Indicates the manufacturer's catalog number so that the medical device can be identified.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.1
LOT	Lot number/ Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labeling, and information to be supplied	5.1.5

	Use-by date	Indicates the date after which the medical device is not to be used.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.4
	Authorized Representative	Indicates the Authorized Representative in the identified country or jurisdiction.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.1.2
	Do not re-use/ Single use only	Indicates a medical device that is intended for one single use only.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.4.2
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.4.3
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.3.7
	In vitro diagnostic medical device	Indicates that a medical device is intended to be used as an in vitro diagnostic medical device	ISO 15223-1 Medical devices – Symbols to be used with medical device labels, labelling, and information to be supplied	5.5.1
	CE Mark European Conformity	Designates that the product labeled Is authorized for sale in European countries.	EU IVDR (EU) 2017/746	

AUTHORIZED REPRESENTATIVE INFORMATION

REVISION 3
Additions: Intended Use, Intended Users, Ergonomics Precautions, Serious Incident Report Contact Information, Glossary of Symbols
Changes: Title change from Product Insert to Instructions for Use. References updated. Contact information.
Deletions: None