

# ID Membranes for Rapid Microbial Identification



## ID Membranes for Rapid Microbial Identification

Growth on Petri dishes is usually the first step to identify microorganisms. Smart, inexpensive membranes can speed up the subsequent ID procedure to 1 to 4 hours.

These substrate-carrying ID membranes, which are simply placed onto the agar surface after routine incubation, allow the identification and differentiation of colonies by means of characteristic color reactions. This economical and rapid identification and confirmation method is suitable for microorganisms from water, food, environmental and clinical samples. It is used in various sectors, including the food, dairy, water and cosmetics industries, pharmaceutical laboratory testing, environmental and sanitary testing, and clinical diagnostics.

#### **Principle**

The membranes contain chromogenic substrates such as ONPG, X-Gal, or X-Glu, and other substrates and indicators which serve as the basis for microbe differentiation by color. The target organisms possess enzyme systems that metabolize the substrates in the membrane, leading to a change of color. The colors can be visually detected on the membrane. This indicates microbial enzymatic activity which serves to identify the genus and species. No reagents or liquids need to be added. If the membrane is removed from the plate after replication of the colony pattern, the plate can be used for further tests or to streak out colonies onto different plates.

### Method workflow

- 1. Inoculation and isolation: Inoculate any general-purpose medium, such as nutrient agar, tryptic soy agar or plate count agar, with your sample. Any surface plating method can be used, for example the spread plate method, quadrant (four or five) streak pattern or T streak method so as to obtain isolated colonies.
- 2. Plate incubation: Incubate the inoculated agar plate at 35 to 37 °C for 18 to 24 hours to grow colonies,
- 3. Colony replication: Take a membrane from the box using sterile forceps (Figure 1) and place it on the surface of the agar (Figure 2). Allow the membrane to adsorb the grown colonies for 30 seconds to 1 minute (Figure 3). Then remove the membrane and incubate it in an empty Petri dish at 35 to 37 °C for 1 to 4 hours, allowing the agar plate to be
- returned to the incubator for further use at a later time. If there is no need to keep the agar plate for later use, it is also possible to either leave the membrane on the agar for the entire 1 to 4 hour incubation period or to place it onto the agar plate's dry (or dried, using sterile cotton) inner side of the lid for incubation.
- **4. Identification:** After membrane incubation, check for colored colonies on the membrane and interpret the results. For further reference the membranes can be autoclaved and then stored or scanned to save the results on a computer.

Specifications: Membranes are sterile and have a diameter of 70 mm.

Storage temperature: 2 to 8 °C (in the dark).



**Fig 1.** A membrane is taken from the box under aseptic conditions. Once removed, it can be marked (arrow) for easier later alignment of the colony patterns on the plate and the membrane.



**Fig 2.** The membrane is placed onto the agar on which colonies have grown.



Fig 3. The colony pattern is replicated onto the membrane.

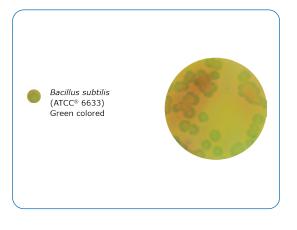
#### **ID** membranes by product

Cat. No.	Product Name	Product Description
78039	Bacillus ID Membrane	For rapid detection and differentiation between various species of <i>Bacillus</i> such as <i>B. subtilis</i> , <i>B. cereus</i> , <i>B. thuringiensis</i> , <i>B. megaterium</i> , <i>B. coagulans</i> , <i>B. pumilus</i> from food, meat, fish, cosmetic and pharmaceutical preparations.

Membrane appearance: Pale pink

Test conditions: Identification observed within 1-4 hours after replication and incubation at 35-37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Bacillus cereus (10876)	Light blue
Bacillus subtilis (6633)	Yellowish green to green
Bacillus thuringiensis (10876)	Light blue

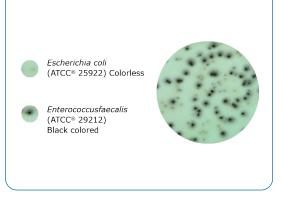


Cat. No.	Product Name	Product Description
01433	Biochemical Esculin ID Membrane (Esculin Test ID Membrane)	For rapid detection of Group D streptococci from food, dairy, water samples and pharmaceutical products etc.

Membrane appearance: Light brown

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37  $^{\circ}$ C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Colorless
Enterococcus faecalis (29212)	Black
Enterococcus faecium (27273)	Black
Staphylococcus aureus (25923)	Colorless

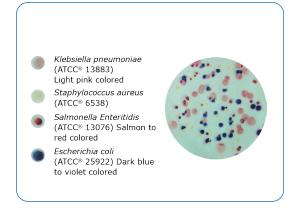


Cat. No.	Product Name	Product Description
19933	Differential Coli- <i>E. coli</i> ID Membrane	For rapid detection of <i>E. coli</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> and <i>Salmonella</i> species in food and environmental samples.

Membrane appearance: White

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37  $^{\circ}$ C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Dark blue to violet
Klebsiella pneumoniae (13883)	Light pink
Pseudomonas aeruginosa (27853)	Colorless
Salmonella Enteritidis (13076)	Salmon to red



Cat. No.	Product Name	Product Description
66964	Differential ID Membrane	For rapid differentiation of lactose fermenting and lactose non-fermenting enteric bacteria from water, food, dairy products, cosmetics, pharmaceutical preparations etc.

Membrane appearance: Pinkish

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Dark pink
Escherichia coli (8739)	Dark pink
Staphylococcus aureus (25923)	Colorless
Salmonella Typhimurium (14028)	Colorless

Flu	orescence under UV (365 r	m)
	Escherichia coli (ATCC® 25922) Dark pink colored	
•	Salmonella Typhimurium (ATCC® 14028) Colorless	

Cat. No.	Product Name	Product Description
73257	DNase ID Membrane	For rapid detection of deoxyribonuclease (DNase) activity of bacteria especially for identification of pathogenic staphylococci.

Membrane appearance: Blue

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Staphylococcus aureus (25923)	Pink zone around the colony, DNase positive

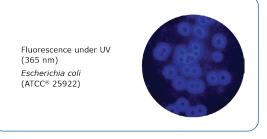
•	,	
	Escherichia coli (ATCC® 25922)	

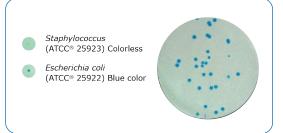
Cat. No.	Product Name	Product Description
03719	Dual Confirmation of E. coli ID Membrane	For rapid detection and confirmation of Escherichia coli in water and food samples, based on chromogenic and fluorogenic methods.

Membrane appearance: White

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37  $^{\circ}$ C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony	Fluorescence under UV light
Pseudomonas aeruginosa (27853)	Colorless	Negative
Escherichia coli (25922)	Blue	Positive
Staphylococcus aureus (25923)	Colorless	Negative
Salmonella Typhimurium (14028)	Colorless	Negative





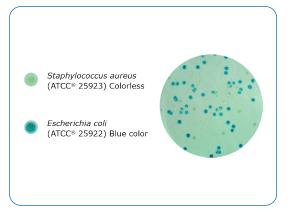
#### ID membranes by product (continued)

Cat. No.	Product Name	Product Description
93009	<i>E.coli</i> Chromogenic ID Membrane	For rapid detection and confirmation of Escherichia coli in water and food samples.

Membrane appearance: White

Test conditions: Identification observed within 1-4 hours after replication and incubation at 35-37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Pseudomonas aeruginosa (27853)	Colorless
Escherichia coli (25922)	Blue
Staphylococcus aureus (25923)	Colorless
Salmonella Typhimurium (14028)	Colorless



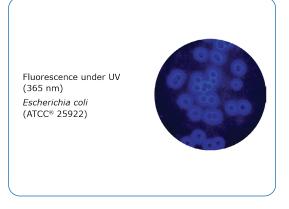
Cat. No.	Product Name	Product Description
06722	E. coli Fluorogenic ID Membrane	For rapid detection and confirmation of Escherichia coli in water and food samples on the basis of fluorogenic emission at 365 nm

Membrane appearance: White

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Pseudomonas aeruginosa (27853)	Negative
Escherichia coli (25922)	Positive
Staphylococcus aureus (25923)	Negative

Negative



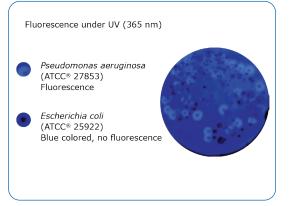
Cat. No.	Product Name	Product Description
51161	<i>Pseudomonas</i> ID Membrane	For rapid detection of <i>Pseudomonas</i> aeruginosa from clinical and nonclinical specimens.

Membrane appearance: White

Salmonella Typhimurium (14028)

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony	Fluorescence under UV light
Escherichia coli (25922)	Colorless	Negative
Pseudomonas aeruginosa (27853)	Colorless	Positive
Enterococcus faecalis (29212)	Colorless	Negative
Klebsiella pneumoniae (13883)	Colorless, mucoid	Negative



Cat. No.	Product Name	Product Description
68122	<i>Salmonella</i> ID Membrane	For rapid detection of <i>Salmonella</i> species from coliforms.

Membrane appearance: White

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Blue
Salmonella Typhimurium (14028)	Light purple
Salmonella Enteritidis (13706)	Light purple
"Klebsiella"pneumoniae (13883)	Colorless, mucoid

	Salmonella Typhimurium (ATCC® 14028) Light purple colored
	Salmonella Enteritidis (ATCC® 13076) Light purple colored
	Klebsiella pneumoniae (ATCC® 13883) Colorless mucoid
•	Escherichia coli (ATCC® 25922) Blue colored

Cat. No.	Product Name	Product Description
77396	Total Coliform ID Membrane	For qualitative detection of coliforms from water, pharmaceutical preparations, dairy and food products.

Membrane appearance: Pink

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37  $^{\circ}$ C, if membrane is placed on on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Dark blue
Enterobacter cloacae (23355)	Salmon to red
Citrobacter freundii (8090)	Salmon to red
Klebsiella pneumoniae (13883)	Light pink

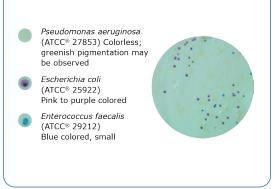
Enterobacter cloacae (ATCC® 23355) Salmon to red colored  Klebsiella pneumoniae (ATCC® 13883) Light pink mucoid colored	Escherichia coli (ATCC® 25922) Dark blue colored	A STATE OF
(ATCC® 13883)	(ATCC® 23355)	
	(ATCC® 13883)	in .

Cat. No.	Product Name	Product Description
39187	Universal Environmental ID Membrane	For rapid detection of <i>Pseudomonas</i> , <i>Enterococcus</i> , <i>E. coli and Salmonella</i> species etc. from environmental samples and samples of clinical origin such as nosocomial samples.

Membrane appearance: Pale Pink

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Pink-purple
Staphylococcus aureus (25923)	Golden yellow
Pseudomonas aeruginosa (27853)	Colorless (greenish pigmentation is observed)
Enterococcus faecalis (29212)	Blue-blue green (small)
Salmonella Typhimurium (14028)	Colorless



#### ID membranes by product (continued)

Cat. No.	Product Name	Product Description
15713	Universal Food Pathogen ID Membrane	For rapid detection of food pathogens such as <i>E. coli</i> , <i>E. coli</i> O157:H7, <i>Staphylococcus aureus</i> , <i>Salmonella</i> , <i>Bacillus</i> , <i>Listeria and Shigella</i> species etc. from various food, dairy, fish, and meat products.

Membrane appearance: Light pink colored

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Purple
Staphylococcus aureus (25923)	Golden yellow
Salmonella Typhimurium (14028)	Colorless
Bacillus cereus (10876)	Light green (big)
Listeria monocytogenes (19111)	Blue-green
Escherichia coli O157:H7 (NCTC 12900)	Purple pink
Shigella flexneri (12022)	Colorless

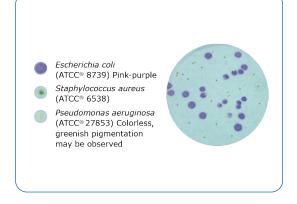
•	Listeria monocytogenes (ATCC® 19111)  Escherichia coli (ATCC® 25922) Purple colored  Escherichia coli 0157:H7 (NCTC 12900) Purple to pink colored  Pseudomonas aeruginosa (ATCC® 27853) Colorless, greenish pigmentation may be observed	

Cat. No.	Product Name	Product Description
00446	Universal Microbial Limit Test Membrane	Recommended for detection of pathogenic microorganisms such as <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>Bacillus and Salmonella</i> species from pharmaceutical preparations, raw materials, cosmetic samples etc.

Membrane appearance: White

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37  $^{\circ}$ C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Pseudomonas aeruginosa (9027)	Colorless
Escherichia coli (8739)	Pink-purple
Staphylococcus aureus (6538)	Green to bluish-green
Salmonella Typhimurium (14028)	Colorless
Salmonella Abony (NCTC 6017)	Colorless



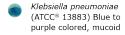
Cat. No.	Product Name	Product Description
30374	UTI ID Membrane (Urinary Tract Infections ID Membrane)	For rapid detection and confirmation of microorganisms mainly causing urinary tract infection, e.g. <i>E. coli, Proteus, Klebsiella, Pseudomonas, S. aureus, and Enterococcus</i> species.

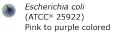
Membrane appearance: White

Test conditions: Identification observed within 1– 4 hours after replication and incubation at 35–37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.

Organisms (ATCC®)	Color of Colony
Escherichia coli (25922)	Pink-purple
Staphylococcus aureus (25923)	Golden yellow
Pseudomonas aeruginosa (27853)	Colorless
Enterococcus faecalis (29212)	Blue-blue green (small)
Klebsiella pneumoniae (13883)	Blue to purple mucoid
Proteus mirabilis (12453)	Light brown

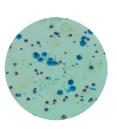






Enterococcus faecalis (ATCC® 29212) Blue colored, small

Staphylococcus aureus (ATCC® 25923) Golden yellow

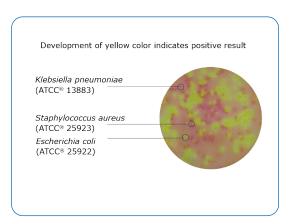


#### **Carbohydrate Fermentation Membranes**

Cat. No.	Product Name	Product Description
05687	Glucose Fermentation Membrane	
52284	Lactose Fermentation Membrane	<ul> <li>For rapid detection of carbohydrate</li> </ul>
39406	Mannitol Fermentation Membrane	fermenting organisms from mixed flora where fermenting organisms will exhibit
41473	Sucrose Fermentation Membrane	yellow color.
92601	Xylose Fermentation Membrane	-

Membrane appearance: Pinkish

Test conditions: Identification observed within 1-4 hours after replication and incubation at 35-37 °C, if membrane is placed on a culture plate of any general medium after 18 hours of growth.



Organisms (ATCC®)	Glucose	Lactose	Mannitol	Sucrose
Citrobacter freundii (8090)	+	+	+	+
Enterobacter aerogenes (13048)	+	+	+	+
Escherichia coli (25922)	+	+	+	_
Klebsiella pneumoniae (13883)	+	+	+	+
Proteus vulgaris (13315)	+	_	_	+
Salmonella Typhimurium (14028)	+	_	+	_
Salmonella Typhi (6539)	+	_	+	_
Serratia marcescens (8100)	+	_	+	+
Shigella flexneri (12022)	+	_	+	_

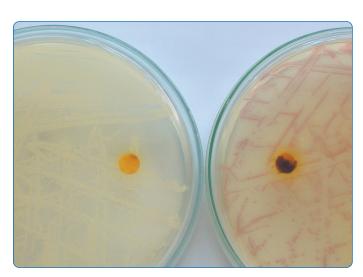
### **Discs & Strips**

Discs and Strips are very helpful and user-friendly for identification and confirmation of microorganisms. They are based on rapid biochemical reagents and reactions, easy to prepare and not expensive.

The strips and discs for differentiation are impregnated with chemical reagents and build intelligent systems for detection of specific abilities and properties from microorganisms. Diverse methods are based on the detection of an enzyme with chromogenic substrate or a complex building reaction. Another possibility is to test the sensitivity to certain inhibitory substances. For having a controlled and save sterilisation of your material we recommend our Sterility Indicators. A well-known example are the oxidase strips to check for cytochrome oxidase activity.

Cat.No.	Description
55876	Adonitol disks
1.13301	Aminopeptidase Bactident™
80372	Arabinose disks
08382	Bacitracin Disks
80507	Bile Esculin Disks
56481	Cellobiose disks
63367	Dextrose disks
05686	DMACA Indole Disks
73044	Dulcitol disks
53901	Fructose disks
89608	Galactose disks
40405	Hippurate Disks
01869	Hippurate Strips Kit
06728	Hydrogen Sulfide Test Strips
04739	Indoxyl Strips
89614	Inositol disks
90058	Inulin disks
78719	Kovac's Reagent Strips
28816	Lactose disks
77653	Maltose disks
94438	Mannitol disks
94445	Mannose disks

Cat.No.	Description
93196	Melibiose disks
51138	Nitrate Reagent Disks Kit
49862	Nitrocefin disks
49940	ONPG Disks
74042	Optochin Disks
40560	Oxidase Strips
70439	Oxidase Test Disks
67886	PYRase Test Strips (Pyrrolidonyl Peptidase Strips)
94226	Raffinose disks
93999	Rhamnose disks
92971	Salicin disks
93998	Sorbitol disks
74146	Sterile disks
05290	Sterility Indicator (Radiation Sterilization)
74041	Sterility Indicator (Steam Sterilization)
94309	Sucrose disks
92961	Trehalose disks
75744	Tributyrin-Strips
89788	V-Factor Disks
08482	X + V Factor Disks
77148	X-Factor Disks
07411	Xylose disks





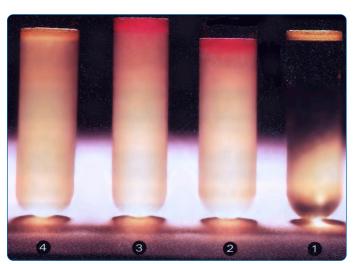


Figure: Kovac Reagent for indoles

## **Biochemical Reagents and Kits**

Biochemical reagents form the basis for liquid biochemical tests to detect, for example, an enzyme activity that is characteristic for a target microorganism. Such tests can often be performed directly on agar plates or liquid media.

Cat.No.	Reagents & Kits			
29333	Barritt's Reagent A	These reagents are used in Voges-Proskauer test for detection of acetoin production by bacterial culture		
39442	Barritt's Reagent B			
40561	beta-Lactamase Testkit	beta-Lactamase test kit is a rapid acidimetric test for detection of beta-lactamase activity of microorganisms. The test is performed in microtitre wells. This test is based on hydrolysis of the beta-lactam ring in benzylpenicillin, which results in the p		
75832	Coagulase Test (Slide)	For the detection of coagulase-negative or -positive organisms  (Staphylococcus aureus).		
74226	Coagulase Test (Tubes)			
49825	DMACA Reagent	For the detection from tryptophanase activity of organisms by verification of indole, which accrued from tryptophan decomposition		
88597	Katalase Test (Hydrogen peroxide 3%)	A reagent to detect the enzymes catalase and peroxidase. Catalase and peroxidase is found in most aerobe bacteria		
67309	Kovac's Reagent for indoles	In the presence of oxygen, some bacteria, like <i>E. coli</i> , are able to split tryptophan into indole and alpha-aminopropionic acid. This reagent is for detecting the indole an identify the indole-positive microorganisms.		
60983	Kovac's Reagent for indoles			
08714	Methyl Red Solution	Some bacteria utilize glucose to form large amounts of acid with the result that the pH value of the medium falls distinct. Other species produce no or less free acid. This difference can be visualized by using methyl red.		
38497	Nitrate Reagent A	The alpha-Naphtylamine and Sulphanilic solution is used to detect nitrate reduction by bacteria. Organisms with nitrate reductase reduce nitrate to nitrite which reacts with sulphanilic acid to form a diazonium salt. With alpha-naphtylamine the salt is converted in a red azo dye.		
39441	Nitrate Reagent B			
73426	Nitrate Reduction Test	Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrite or nitrogenous gases. The reduction of nitrate may be coupled to anaerobic respiration in some species.		
07689	O'Meara's Reagent	The reagent is used in Voges-Proskauer test for the detection of acetoin production by bacterial cultures.		
07345	Oxidase Reagent acc. Gaby-Hadley A	These reagents are used for detection of oxidase activity of bacterial culture according Gaby-Hadley.		
07817	Oxidase Reagent acc. Gaby-Hadley B			
18502	Oxidase Reagent acc. Gordon-McLeod	The reagent is used for detection of oxidase activity of a bacterial culture.		
80353	TDA Reagent	For identification of Proteus species by detection of tryptophan deaminase activity.		

## Millipore®

Preparation, Separation, Filtration & Monitoring Products

Merck KGaA Frankfurter Strasse 250 64293 Darmstadt, Germany

SigmaAldrich.com/IDmembranes

#### To place an order or receive technical assistance

Order/Customer Service: SigmaAldrich.com/order Technical Service: SigmaAldrich.com/techservice Safety-related Information: SigmaAldrich.com/safetycenter

© 2021 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. Merck, the vibrant M and Millipore are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

MK\_BR7871EN Ver. 1.0 36068 06/2021