Agars
Bacto™ Agar • Agar, Grade A • Agar, Granulated Agar, Technical • Agar, Noble • Agarose • Agar, Select

Intended Use

Bacto™ Agar is a solidifying agent in which extraneous matter, pigmented portions and salts have been reduced to a minimum. **Bacto** Agar is used in preparing microbiological culture media.

Agar, Grade A is a high-grade agar, specially processed for microbiological purposes. It is routinely used as a solidifying agent in microbiological media.

Agar, Granulated is a solidifying agent used in preparing microbiological culture media.

Agar, Technical is a solidifying agent used in preparing microbiological culture media. Although Agar, Technical has wider quality control parameters than other bacteriological agars, solubility, gelation temperature and solidity are carefully monitored to permit its use.

Agar, Noble is a solidifying agent that is essentially free of impurities. It is used in electrophoretic and nutritional procedures and in preparing microbiological culture media when increased purity is required.

Agarose is a complex galactose polysaccharide of near neutral charge. It is specially prepared and is intended mainly for use in gel electrophoresis.

Agar, Select is recommended for molecular genetics testing.

Summary and Explanation

Agar is a phycocolloid extracted from a group of red-purple marine algae (Class Rhodophyceae) including *Gelidium, Pterocladia* and *Gracilaria*. *Gelidium* is the preferred source for agars. Impurities, debris, minerals and pigment are reduced to specified levels during manufacture.

Agar was first suggested for microbiological purposes in 1881 by Fannie Hesse.[1,2] By the early 1900s, agar became the gelling agent of choice over gelatin because agar remains firm at growth temperatures for many pathogens. Agar is also generally resistant to a breakdown by bacterial enzymes. The use of agar in microbiological media significantly contributed to the advance of microbiology, paving the way for pure culture isolation and study.

Agar is a gel at room temperature, remaining firm at temperatures as high as 65°C.[3] Agar melts at approximately 85°C, a different temperature from that at which it solidifies, 32-40°C. This property is known as hysteresis. Agar is generally resistant to shear forces; however, different agars may have different gel strengths or degrees of stiffness.

Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller quantities (0.05-0.5%) are used in media for motility studies (0.5% w/v) and for growth of anaerobes (0.1%) and microaerophiles.[3]

The use of small amounts of agar in media for sterility testing was recommended by Falk et al.[4] and has been incorporated into Fluid Thioglycollate Medium for sterility testing by standard procedures.[5]

Specifications for bacteriological grade agar include good clarity, controlled gelation temperature, controlled melting temperature, good diffusion characteristics, absence of toxic bacterial inhibitors and relative absence of metabolically useful minerals and compounds.

Principles of the Procedure

Bacto Agar is optimized for beneficial calcium and magnesium content. Detrimental ions such as iron and copper are reduced. **Bacto** Agar is recommended for clinical applications, auxotrophic studies, bacterial and yeast transformation studies and bacterial molecular genetics applications.[6,7]

Grade A Agar is a select grade of agar containing essential minerals for bacterial growth. When utilized as an ingredient, most media formulations demonstrate improved growth and test reactions.

Granulated Agar is qualified for culturing recombinant strains of *Escherichia coli* (HB101) and *Saccharomyces cerevisiae*. Agar, Granulated may be used for general bacteriological purposes where clarity is not a strict requirement.

Technical Agar is suitable for many bacteriological applications. This agar is not highly processed, has broader technical specifications than other agars and is not recommended for growth of fastidious organisms.

Noble Agar is extensively washed and bleached. This agar should be used for applications where extreme clarity and high purity are required. Noble Agar is suitable for immunodiffusion, some electrophoretic applications, and as a substrate for mammalian or plant tissue culture.

Agarose is the low sulfate, neutral gelling fraction of agar. During the fractionation of agar, the agarose-portion is separated from the highly charged polysaccharides (high sulfate, nongelling portion), purified and dried. Because of its method of preparation, Agarose is considerably purer than the special kinds of agar, with respect to ionic groups, rendering it more valuable for gel electrophoresis.[8] In addition to high chemical purity, Agarose must exhibit certain physical properties; e.g., high gel strength and high gel clarity.[8] The suggested concentration for use is 0.5-1.2%.

Select Agar is a key ingredient used in molecular genetics work for determining bacteriophage lambda titers.

User Quality Control

Identity Specifications

	BACTO™ AGAR	BBL™ AGAR, GRADE A	DIFCO™ AGAR, GRANULATED	DIFCO™ AGAR, TECHNICAL	DIFCO™ AGAR, NOBLE	BBL™ AGAROSE	BBL™ AGAR, SELECT
Dehydrated Appearance	Very light beige to light beige, free-flowing, homogeneous granules.	Light to medium, yellow-cream to cream-tan, homogenous, free of extraneous material.	Very light to light beige, free-flowing, homogeneous granules.	Very light to medium beige, free-flowing, homogeneous.	White to off-white, free-flowing, homogeneous fine granules.	White to light tan, homogeneous, free of extraneous material.	Light cream to tan, homo-geneous, free of extraneous material.
Solution Concentration	1.5%	1.5%	1.5%	1.5%	1.5%	1.0%	1.5%
Solution Appearance*	Solution is very light amber, very slightly to slightly opalescent, may contain a small amount of black particles.	Solution is colorless to tan, slightly hazy (minute to small cream particles may be present).	Solution is very light amber, very slight to slightly opalescent.	Solution is very light to medium amber, slightly opalescent to opalescent.	Solution is colorless to pale yellow, clear to very slightly opalescent.	Solution is colorless.	Solution is pale to light yellow to tan, clear to moderately hazy (minute to fine cream particles may be present).
pH at 25°C	N/A	5.5-7.5	N/A	N/A	N/A	6.1-7.1	5.5-7.5
Loss on Drying (LOD)	16-20%	5-11%	≤ 20%	≤ 20%	≤ 20%	≤ 10%	5-10%
Ash[5]	≤ 6.5%	3.0-6.5%	≤ 6.5%	≤ 6.5%	≤ 2%	≤ 1.0%	2.0-6.5%
Calcium µg/g (ppm)	300-3,000 ppm	N/A	≤ 300-2,500 ppm	≤ 3,000 ppm	≤ 1,000 ppm	N/A	N/A
Magnesium µg/g (ppm)	50-1,000 ppm	N/A	≤ 50-1,000 ppm	≤ 1,300 ppm	≤ 400 ppm	N/A	N/A
Melting Point	83-89°C	80-90°C	83-89°C	≥ 85°C	≥ 85°C	N/A	80-90°C
Gelation Point	32-39°C	33-38°C	32-39°C	32-39°C	32-39°C	N/A	33-38°C
Agar Gel Electrophoresis	N/A	N/A	N/A	N/A	Satisfactory	Satisfactory	N/A

**Soluble in purified water upon boiling.*

Cultural Response

Prepare the agar formulation of Nutrient Broth or LB Broth, Miller by adding 1.5% agar. Inoculate with 10^2-10^3 CFU of the indicated test organisms and incubate at 35 ± 2°C for 18-24 hours (18-72 hours for LB Broth, Miller). Record recovery.

	BACTO™ AGAR	DIFCO™ AGAR, GRANULATED	DIFCO™ AGAR, TECHNICAL	DIFCO™ AGAR,* NOBLE
Nutrient Broth with:				
Escherichia coli ATCC™ 25922	Good		Good	Good
Staphylococcus aureus ATCC™ 25923	Good		Good	Good
LB Broth, Miller with:				
Escherichia coli ATCC™ 33694 (HB101)		Good		
Saccharomyces cerevisiae ATCC™ 9763		Good		

**To evaluate for growth in tissue culture, prepare TC Medium 199 with 10% fetal calf serum and 0.5% Noble Agar. Adjust pH to 7.4-8.0. Inoculate tissue culture flasks with Vero cells and observe for attachment and division.*

Bacto™ Agar

BBL™ Agar, Grade A

This product is tested for satisfactory performance as plain **Trypticase**™ Soy Agar. Spread plates are inoculated in duplicate with serial dilutions of *Neisseria meningitidis* (ATCC™ 13090), *Streptococcus pneumoniae* (ATCC™ 6305) and *Streptococcus pyogenes* (ATCC™ 49117) such that one dilution contains 30-300 CFU/mL. Plates are incubated at 35 ± 2°C for 1 day with 3-5% CO_2. A satisfactory result corresponds to colony counts that are within 1.2 logs of an acceptable control lot.

BBL™ Agar, Select

This product is tested as NZC Bottom Agar and NZC Top Agar and tested for satisfactory propagation of bacteriophage lambda Charon 30 utilizing *Escherichia coli* ATCC 33526 (K802). To prepare NZC agars, add, per liter of purified water: Casitone, 10 g; Casamino Acids, 1.0 g; Sodium Chloride, 5.0 g; Magnesium Chloride (anhydrous), 0.94 g; for NZC Bottom Agar, add 9.0 of Select Agar; for NZC Top Agar, add 6.0 g of Select Agar.

Procedure

See appropriate references for specific procedures using **Bacto**™ Agar, Grade A Agar, Granulated Agar, Technical Agar, Noble Agar, Agarose or Select Agar.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Hesse. 1894. Mitt. a.d. Kaiserl. Gesh. Berlin 2:182.
2. Hitchens and Leikind. 1939. J. Bacteriol. 37:485.
3. Selby and Selby. 1959. Agar. *In* Whister (ed.), Industrial gums. Academic Press Inc., New York, N.Y.
4. Falk, Bucca and Simmons. 1939. J. Bacteriol. 37:121.
5. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
6. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, N.Y.
7. Schiestl and Geitz. 1989. Current Genetics *16*:339.
8. Guiseley and Renn. 1975. Agarose: purification, properties, and biomedical applications. Marine Colloids, Inc. Rockland, Maine.

Typical Analyses

	BACTO™ AGAR	BBL™ AGAR, GRADE A	DIFCO™ AGAR, GRANULATED	DIFCO™ AGAR, TECHNICAL	DIFCO™ AGAR, NOBLE	BBL™ AGAROSE	BBL™ AGAR, SELECT
Physical Characteristics							
Concentration (%)	1.5	1.5	1.5	1.5	1.5	1.0	1.5
Ash (%)	3.6	3.0-6.5	3.4	4.1	1.3	< 1.0	2.0-6.5
Clarity (NTU)*	4.3	< 10	5.3	26.2	3.7	< 10	N/A
Color (430 nm, adsorbance)	N/A	< 0.2	N/A	N/A	N/A	< 0.2	N/A
Loss on Drying (%)	17.3	< 10	12.2	18.2	16.0	< 10	N/A
pH	6.5	5.5-7.5	6.6	6.9	5.7	6.0-7.0	5.5-7.5
Gel Strength (g/cm^2)	600	600-800	560	613	700	800-1200	N/A
Gelation Point (°C)	35	35-39	35	36	35	35-39	33-38
Melting Point (°C)	88	80-90	88	88	87	80-90	80-90
Resistivity (ohms)	N/A	N/A	N/A	N/A	N/A	> 50,000	N/A
-m_r (electrophoretic)**	N/A	N/A	N/A	N/A	≤ 0.55	< 0.25	N/A
Inorganics (%)							
Calcium	0.179	0.23	0.133	0.110	0.015	0.03	N/A
Chloride	0.021	N/A	< 0.005	0.172	< 0.050	N/A	N/A
Cobalt	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Copper	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Iron	0.002	< 0.0060	0.003	0.002	< 0.001	< 0.0050	N/A
Lead	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Magnesium	0.068	0.10	0.041	0.093	0.002	0.01	N/A
Manganese	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Nitrate	< 0.005	N/A	< 0.005	< 0.005	< 0.050	N/A	N/A
Phosphate	< 0.005	0.02	0.010	0.015	< 0.050	0.08	N/A
Potassium	0.121	0.03	0.079	0.124	0.022	0.015	N/A
Sodium	0.837	1.8	0.776	0.932	0.335	< 0.1	N/A
Sulfate	1.778	N/A	1.710	0.367	0.663	N/A	N/A
Sulfur	0.841	0.7	0.868	0.646	0.333	0.1	N/A
Tin	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Zinc	< 0.001	N/A	< 0.001	< 0.001	< 0.001	N/A	N/A
Biological Testing (CFU/g)							
Spore Count	< 1,000	≤ 20	< 1,000	4,300	< 1,000	N/A	N/A
Standard Plate Count	< 1,000	N/A	< 1,000	2,725	< 1,000	N/A	N/A

**Nephelometric turbidity units (NTU).*
***Unit of relative electroendosmosis.*

Availability

Bacto™ Agar

Cat. No. 214050 Dehydrated – 100 g
214010 Dehydrated – 454 g
214030 Dehydrated – 2 kg
214040 Dehydrated – 10 kg

BBL™ Agar, Grade A

Cat. No. 212304 Dehydrated – 454 g

Difco™ Agar, Granulated

Cat. No. 214530 Dehydrated – 500 g
214510 Dehydrated – 2 kg
214520 Dehydrated – 10 kg

Difco™ Agar, Technical

Cat. No. 281230 Dehydrated – 500 g
281210 Dehydrated – 2 kg

Difco™ Agar, Noble

Cat. No. 214220 Dehydrated – 100 g
214230 Dehydrated – 500 g

BBL™ Agarose

Cat. No. 212272 Dehydrated – 500 g

BBL™ Agar, Select

Cat. No. 299340 Dehydrated – 500 g
299341 Dehydrated – 5 lb (2.3 kg)