

Super Broth (Animal Free)

Intended Use

Select APS™ Super Broth is a molecular genetics medium used to grow *Escherichia coli* to a high cell density.

Summary and Explanation

The Select Alternative Protein Source (APS) media were designed as alternatives to classical animal-based media for the maintenance and propagation of *Escherichia coli* strains in molecular genetics procedures. Select APS media are manufactured from animal-free ingredients in order to minimize the risk of bovine spongiform encephalopathy in culture media containing animal, and especially bovine, materials.

Select APS Super Broth is based on the Terrific Broth formulation designed by Tartof and Hobbs.¹ The medium was developed for ease of use to improve yield of plasmid-bearing *E. coli* strains over that of LB Broth. The 1.2% tryptone in Terrific Broth was replaced with the animal-free component, soy hydrolysate, in the same concentration in Select APS Super Broth. The 2.4% yeast extract and recommended addition of 5 mL/L glycerol is the same for Select APS Super Broth as is used in classical Terrific Broth. The buffering system, 1.14% dipotassium phosphate and 0.17% monopotassium phosphate, is altered from that of classical Terrific Broth. The formulation for Select APS Super Broth does not contain glucose, thus preventing acetate build-up in the fermentation process.²

Select APS Super Broth was used in the production of purified recombinant human uteroglobin for the treatment of inflammatory and fibrotic conditions.³ In addition, the medium was used in the process development and analysis of a preerythrocyte-stage protein-based vaccine for *Plasmodium falciparum*.⁴ Select APS Super Broth was also used in a study to show that heterologous

protein expression was enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host.⁵

Principles of the Procedure

Soy hydrolysate provides nitrogen and carbon compounds for bacterial metabolism. Yeast extract supplies vitamins, amino acids and trace elements which enhance bacterial growth and plasmid yield. The phosphate buffering system prevents cell death caused by pH drop. Glycerol is added as a carbon and energy source which, unlike glucose, is not fermented to acetic acid.

Formula

Difco™ Select APS™ Super Broth

Approximate Formula* Per Liter	
Soy Hydrolysate.....	12.0 g
Yeast Extract	24.0 g
Dipotassium Phosphate.....	11.4 g
Monopotassium Phosphate.....	1.7 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 49.1 g of the powder and 5 mL of glycerol in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.^{6,7}

Expected Results

Growth is evident by the appearance of turbidity.

References

1. Tartof and Hobbs. 1987. Bethesda Research Laboratories Focus. 9:12.
2. Swartz. 2001. Curr. Opin Biotechnol. 12:195.
3. World Intellectual Property Organization. 2003. WO/2003/003979 (Claragen, Inc.) 2003-1-16. World Intellectual Property Organization, Geneva, Switzerland.
4. Hillier, Ware, Barbosa, Angov, Lyon, Heppner and Lanar. 2005. Infect. Immun. 73:2109.
5. Angov, Hillier, Kincaid and Lyon. 2008. PLoS ONE. 3(5):e2189. Doi: 10.1371/journal.pone.0002189.
6. Sambrook, and Russell. 2001. Molecular cloning, a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 2002. Short protocols in molecular biology, 5th ed. John Wiley & Sons, Inc., Hoboken, N.J.

Availability

Difco™ Select APS™ Super Broth

Cat. No.	212485	Dehydrated – 500 g
	212486	Dehydrated – 10 kg

User Quality Control

Identity Specifications

Difco™ Select APS™ Super Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.9% solution, soluble in purified water. Solution is medium to dark, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Medium to dark, yellow to tan, clear to moderately hazy.
Reaction of 4.9% Solution at 25°C:	pH 6.8 – 7.5

Cultural Response

Difco™ Select APS™ Super Broth

Prepare the medium per label directions. Inoculate and incubate at 35-37°C, 250 rpm for 12 hours.

ORGANISM	ATCC™	RECOVERY
<i>Escherichia coli</i>	700790	Good