Preparing Agarose for use in Cell Culture Applications

Introduction

Agarose has unique properties that make it especially useful in cell culture media applications. Agarose can form stable gels at concentrations as low as 0.3% (w/v), has very low ionic concentrations and is free of contaminating impurities that may affect the growth characteristics of cells in culture. Agarose could be used directly without supplementation for plaque assays ensuring high cell variability. The use of agarose is suggested for media where the absence of a nutrient is mandatory.

Advantages

- Highly purified, eliminating contaminants
- Excellent optical transparency which enhances colony observation
- Gelling temperatures below 28°C allowing manipulation of cells in solution
- Can be used to produce a 'defined' media system

Applications

- Cell culture media
  - Mammalian
  - Plant
  - Bacterial
  - Viral
- Overlay assays
- Plaque hybridization assays
- Soft agarose hybridoma cloning
- Attachment dependent cell culture
- Colony lift assays
- Analysis of matrix and cell matrix interactions

The concentration and type of agarose to use largely depends on your cell system, the application, and the lot specific gel strength of the agarose. There are three types of agarose, which can be used for cell culture media, each having unique properties making one more suitable for a given application than another.

- **Biozym LE Agarose –** A standard melting and gelling temperature Agarose which can be used as a solid medium to support cell growth by supplementation with growth factors and nutrients, top agar enriched with magnesium for baculovirus screening, substrate for bacterial growth and for colony lifts.

- **Biozym Plaque Agarose –** A low gelling temperature agarose (28°C) that remains a liquid at 37°C allowing the manipulation of cells within the solution. Biozym Plaque Agarose can be used as a semi-solid media for anchorage independent assays, plaque assays or overlays. Biozym Plaque Agarose has also been found to be very effective as a medium for protoplast culture.

- **Biozym Prep Agarose –** Unique ultra-low gelling temperature (15°C) and gel strength agarose (>75 g/cm^2). Biozym Prep Agarose is ideal for hybridoma cloning. Cells can be recovered from the gel by increasing the temperature slightly allowing transfer to a viable cell suspension for subsequent growth in liquid medium.

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Suggested agarose products and concentration guidelines

The table below provides general guidelines on agaroses and gel concentrations for given applications. General Guidelines and specific information pertaining to a given cell type and application can be obtained from the current literature and *Cell Biology: A Laboratory Manual.*

Application	Biozym LE Agarose	Biozym Plaque Agarose	Biozym Prep Agarose
Mammalian cell culture	0.3% - 1%	0.25% - 1.5%	
Baculovirus screening		0.3% - 0.6%	
Bacterial culture	0.7% - 1.0%	1.0%	
Colony Lifts	0.7% - 1.0%	1.0%	
Anchorage independent assays	0.25% - 0.5%		
Plaque assays	0.3% - 1%	0.50% - 1.5%	
Overlays	0.3% - 0.6%	0.3% - 1%	
Protoplast culture		0.35% - 0.7%	
Hybridoma cloning			0.8% - 1.5%
Virus Enumeration			1%
Cell matrix interactions		1.0% - 3.0%	
Attachment Dependent Cell culture		0.6%	
In vitro cloning – mammalian	0.5% - 2%		1% - 4%

NOTE: Higher agarose concentration gels can affect and possibly restrict cell proliferation. Optimal gel concentrations should be determined for each culture system on a case-by-case basis.
In some cases, such as hybridoma cloning using SeaPrep Agarose it is advisable to sample different lots of agarose for the desirable gel strength qualities.

Agarose preparation

When preparing agarose for cell culture work, it is always best to prepare the agarose in water suitable for cell culture and separate from any growth media or nutrients. Agarose solutions and media solutions should be prepared at 2X concentrations (i.e., if desired final agarose concentration is 0.6%, prepare a 1.2% agarose solution), autoclaved separately, and aliquoted into useable aliquots.

Procedure for Autoclaving Agarose

1. Choose a flask that is 2 - 4 times the volume of the solution.
2. Add water to the flask.
3. Sprinkle in the pre-measured agarose powder at a 2X final agarose concentration.
4. Cover the flask with aluminum foil.
5. Place the flask in the autoclave.
6. Sterilize the agarose by autoclaving for 10 minutes at 120°C at 1 atm.
If using Biozym Prep Agarose, autoclave for no longer than 5 minutes.

NOTE: Agarose may lose gel strength when exposed to longer periods in the autoclave.

7. Once the agarose solution has cooled, aliquot into useable aliquots and store at 4°C prior to use.

Tips for Cell Culture Applications

General Procedure for Using Agarose in Culture Medium

1. Remelt the agarose by placing in a hot water bath or microwave.

2. Allow the agarose solution to cool to 37°C.

3. Prewarm the 2X media solution to 37°C.

4. Mix equal volumes of the sterile 2X agarose solution with sterile 2X media containing growth factors and nutrients.

5. Cast the agarose/media solution into plates or sterile culture tubes.

6. Allow the agarose solution to gel for 20 minutes if using as a feeder or overlay, or maintain the solution at 37°C, if using as a liquid culture.

> **NOTE:** A solution containing 1% Biozym Plaque Agarose will stay liquid for approximately 18 hours. The amount of time an Agarose solution will stay in a liquid state at 37°C, largely depends on the agarose concentration (increased agarose concentrations will decrease the time the solution stays in a liquid state), the age of the agarose and the particular lot of agarose you are using. When purchasing a new lot of agarose, we recommend you test this prior to culturing cells.

References

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