Agarose for the Separation of GeneAmp® PCR Products

Protocol

Applied Biosystems

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Product and Protocol Overview

About the Product

Agarose for the Separation of GeneAmp® PCR Products is a composite that forms gels that have high strength and flexibility. Because of its low viscosity, this agarose is easy to pour and can be used at high concentrations to produce high-resolution gels.

Agarose for Separation of GeneAmp PCR Products is custom blended for Applied Biosystems by Cambrex Bio Science Rockland, Inc., for the separation of polymerase chain reaction (PCR) products.

Product Features

- Agarose for the Separation of GeneAmp PCR Products gels at 38 °C or less and melts at 90 °C or less.
- Gels cast from Agarose for Separation of GeneAmp PCR Products are compatible with standard electrophoresis equipment.
- Agarose for Separation of GeneAmp PCR Products can distinguish fragments as small as 10 base pairs (Saiki *et al.*1988)[1] and is ideal for resolution of electrophoresed DNA fragments below one kb.
- Agarose for the Separation of GeneAmp PCR Products has been tested for the absence of DNase activity, RNase activity, and for low DNA binding.

Because of these features, Agarose for the Separation of GeneAmp PCR Products is guaranteed to support reliable nucleic acid separations.

About This Protocol

This protocol describes how to:

- Determine the optimal agarose concentration
- Dissolve the agarose
- Cast an agarose gel
- Load an agarose gel

1. Saiki, R.K., Gelfand, D.H., Stoffel, S., *et al.* 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–491.

Product Contents and Storage

Product Contents

This agarose is available in one size.

Item	Quantity	Part Number
Agarose for Separation of GeneAmp PCR Products	100 g	N930-2774
Agarose for Separation of GeneAmp PCR Products Protocol	1	9938732

Storing the Product

Agarose for Separation of GeneAmp PCR Products can be stored at ambient temperature.

Required Materials

Overview

This section describes the materials necessary for making an agarose gel, electrophoresing the gel and recovering the DNA after electrophoresis.

Materials Required but Not Included

The following materials are required but not supplied:

Item	Source
Submarine or vertical electrophoresis apparatus	Major laboratory supplier (MLS)
Autoclavable bottle, flask, or beaker	MLS
Stirring rod	MLS
Microwave or boiling water bath	MLS
Stirring hot plate	MLS
Laboratory balance	MLS
Long-wave UV light box	MLS
One of the following buffers: TAE; 40-mM Tris-Acetate, 1-mM EDTA, pH 8.0 TBE; 89-mM Tris, 89-mM Borate, 2-mM ETDA, pH 8.0	 MLS MLS
Ethidium bromide	MLS
DNA purification kit	MLS

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing).
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

Step	Action
1	Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2	In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3	Find the document of interest, right-click the document title, then select any of the following: ♦ **Open** – To view the document ♦ **Print Target** – To print the document ♦ **Save Target As** – To download a PDF version of the document to a destination that you choose
4	To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5	After you enter the required information, click **View/Deliver Selected Documents Now**.

Determining the Agarose Concentration

General Recommendations

- The final agarose concentration that you choose should depend on the size of the products you are separating.
- The best separation of DNA fragments from 10 to 1000 bp is obtained on a 4% agarose gel in TAE buffer or a 3% gel in TBE buffer. Refer to "Materials Required but Not Included" on page 3 for the buffer components.
- TBE buffer makes a gel appear to have smaller pores so that for a given length of time, the mobility of DNA is less than it would be in TAE buffer.

Note Gels with a concentration of agarose 2% or less are difficult to handle.

Suggested Agarose Concentrations

Size Range (in base pairs)	Final Agarose Concentration	
	TAE (% w/v)	TBE (%w/v)
500–1000	3	–
100–500	4	3
10–100	6	5

Electrophoresis Buffers

The recipes for 1 L of 1X TAE and 1X TBE are provided below.

⚠ CAUTION **CHEMICAL HAZARD. Tris** (hydroxymethyl) aminomethane (TRIS) may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ WARNING **CHEMICAL HAZARD. Boric acid** is a hazardous chemical that is harmful if ingested, inhaled, or absorbed through the skin and can be irritating to the eyes, respiratory system, and skin. Handling boric acid while pregnant brings possible risk to the unborn child. Prolonged or repeated exposure can potentially impair fertility. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ WARNING **CHEMICAL HAZARD. Glacial acetic acid** is a corrosive and flammable chemical that causes severe burns and is harmful when inhaled or swallowed. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ CAUTION CHEMICAL HAZARD. EDTA may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1X TAE[1]

Dissolve the reagents in 900 mL of water and adjust the pH to 8.0–8.5. After adjusting the pH, bring the solution up to a final volume of 1000 mL.

Component	Quantity/1000 mL	Final Concentration
Tris base	4.84 g	40 mM tris-acetate
Glacial acetic acid	1.14 mL	
$Na_2EDTA\text{-}2H_2O$	744 mg	2 mM

1X TBE[b]

Dissolve the reagents in 900 mL of water and adjust the pH to 8.0. After adjusting the pH, bring the solution up to a final volume of 1000 mL.

Component	Quantity/1000 mL	Final Concentration
Tris base	10.8 g	89 mM
Boric acid	5.5 g	89 mM
0.50 M EDTA	4 mL	2 mM

1. Adapted from; Commonly used reagents and equipment. In *Current Protocols in Molecular Biology,* Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. eds., 1999, John Wiley and Sons Inc.

Dissolving the Agarose

Overview

You can use either of the following methods for dissolving the Agarose for the Separation of GeneAmp PCR Products:

- Heating the agarose/buffer mixture in a boiling water bath
- Heating the agarose/buffer mixture in a microwave oven

⚠ WARNING **PHYSICAL INJURY HAZARD**. **Hot Surface and Liquid.** Use care when working with boiling water baths to avoid being burned by hot components and boiling liquid.

⚠ WARNING **PHYSICAL INJURY HAZARD**. **Hot Liquid**. Exercise caution when using the microwave method. Agarose can become superheated and sudden boiling can occur when you remove the agarose solution from the microwave oven.

In both methods, you add the preweighed agarose to the chosen buffer to give the final required concentration. Use an autoclavable bottle, Erlenmeyer flask, or a beaker for this procedure, but do not use metal caps or bottles that have sealed caps.

The microwave method offers the advantage of being fast and less likely to burn the agarose.

Dissolving the Agarose in a Boiling Water Bath

To dissolve the agarose using a boiling water bath:

Step	Action
1	Weigh out the appropriate amount of agarose.
2	Tare an autoclavable bottle, flask, or beaker containing a magnetic stirring bar.

To dissolve the agarose using a boiling water bath: *(continued)*

Step	Action
3	Add a measured volume of buffer to the vessel. Make sure the buffer is at room temperature and has a pH of 7.0 or greater. **Note** TAE or TBE buffers are recommended. **⚠ CAUTION CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION CHEMICAL HAZARD. Tris** (hydroxymethyl) aminomethane (TRIS) may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ WARNING CHEMICAL HAZARD**. **Boric acid** is a hazardous chemical that is harmful if ingested, inhaled, or absorbed through the skin and can be irritating to the eyes, respiratory system, and skin. Handling boric acid while pregnant brings possible risk to the unborn child. Prolonged or repeated exposure can potentially impair fertility. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Slowly sprinkle the previously weighed agarose into the buffer while constantly stirring to prevent lumps from forming.
5	Let the agarose particles disperse completely, using a glass rod to break up and disperse any clumps.
6	Weigh the vessel and its contents.
7	Cover the vessel with parafilm or plastic wrap. Make a small hole in the seal for a steam vent.
8	Place the suspension in a boiling water bath. Stir for 10 min or until the agarose has completely dissolved.
9	Hold the vessel up to a light to see if there are any undissolved particles. If you see any undissolved particles, continue to boil and stir the mixture. **Note** The mixture may turn slightly yellow. This is normal.

To dissolve the agarose using a boiling water bath: *(continued)*

Step	Action
10	When the agarose has completely dissolved, weigh the contents of the vessel. Add warm distilled water to compensate for any losses that occurred during boiling. Generally, less than 1 mL of water will be lost.
11	Cool the mixture to 50 °C for gels with concentrations greater than 1% agarose. The agarose is now ready for casting.

Dissolving the Agarose in a Microwave

IMPORTANT Do not use this method for gels with agarose concentrations greater than 6%.

⚠ WARNING **PHYSICAL INJURY HAZARD**. **Hot Liquid**. Exercise caution when using the microwave method. Agarose can become superheated and sudden boiling can occur when you remove the agarose solution from the microwave oven.

To dissolve the agarose using a microwave oven:

Step	Action
1	Weigh out the appropriate amount of agarose.
2	Tare an autoclavable vessel containing a magnetic stirring bar so you can adjust the volume by weight. **Note** Make sure that the vessel can hold four-times the volume of solution.

To dissolve the agarose using a microwave oven: *(continued)*

Step	Action
3	Add a measured volume of buffer to the vessel. Make sure the buffer is at room temperature and has a pH of 7.0 or greater. **Note** TAE or TBE buffers are recommended. **⚠ CAUTION CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION CHEMICAL HAZARD. Tris** (hydroxymethyl) aminomethane (TRIS) may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ WARNING CHEMICAL HAZARD**. **Boric acid** is a hazardous chemical that is harmful if ingested, inhaled, or absorbed through the skin and can be irritating to the eyes, respiratory system, and skin. Handling boric acid while pregnant brings possible risk to the unborn child. Prolonged or repeated exposure can potentially impair fertility. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Slowly sprinkle the previously-weighed agarose into the vessel while constantly stirring to prevent lumps from forming.
5	Let the agarose particles disperse completely. Use a glass rod to break up and disperse any clumps. **Note** Stir for a minimum of 3 min.
6	Remove the magnetic stirring bar.
7	Tare the vessel and its contents.
8	Cover the vessel with parafilm or plastic wrap, Make a small hole in the seal for a steam vent. **Note** If the vessel is capped, make sure the cap is loose.

To dissolve the agarose using a microwave oven: *(continued)*

Step	Action
9	Place the vessel in a microwave oven and heat it for 2 min on the medium power setting. **⚠ WARNING PHYSICAL INJURY HAZARD. Hot Liquid.** Exercise caution when using the microwave method. Agarose can become superheated and sudden boiling can occur when you remove the agarose solution from the microwave oven.
10	Remove the vessel from the microwave oven and gently swirl the contents of the vessel to suspend any settled powder and pieces of gel.
11	Place the vessel in the microwave oven. Heat it for 1–2 min on the high power setting, or until the agarose solution comes to a boil.
12	Remove the vessel from the microwave oven. Gently swirl the contents of the vessel to thoroughly mix the agarose solution.
13	Check the solution for undissolved particles and, if necessary, reheat it in the microwave oven for 1–2 min. **Note** The agarose/buffer mixture may turn slightly yellow. This is normal.
14	Weigh the vessel and its contents and add warm, distilled water to obtain the previous weight. Generally less than 1 mL is lost during the process.
15	Cool the agarose solution to 50 °C before you pour the gel. The gel is now ready for casting.

Casting, Loading, and Staining an Agarose Gel

Overview

Agarose for the Separation of GeneAmp PCR PRoducts can be cast into the same electrophoresis chambers (regular or mini) used with other agaroses.

General Recommendations

- For the sharpest resolution, use a narrow-toothed comb, approximately 1 mm wide. The amount of DNA loaded per well affects the resolution.
- Agarose gels are typically 3 mm thick. Thinner gels are more difficult to handle.
- To visualize small molecular weight fragments after staining, the lane must often be overloaded

Loading and Staining a Submarine Gel

The following procedure is for 3-mm thick horizontal or submarine gels.

To load a horizontal gel:

Step	Action
1	Let the gel set (a 4% gel requires about 30 min to set).
2	Overlay the gel with a 2–3-mm layer of 1X TAE or TBE buffer.
3	Slowly ease the comb out of the gel. Remove the ends and add more buffer.
4	Run the gel submerged under a thin layer of buffer. See the table in "General Electrophoresis Conditions" on page 15 for general electrophoresis conditions and run lengths.
5	Soak the gel in buffer containing 1 µg/mL of ethidium bromide for 20 min. **⚠ WARNING CHEMICAL HAZARD. Ethidium bromide** causes eye, skin, and respiratory tract irritation and is a known mutagen (*i.e.*, it can change genetic material in a living cell and has the potential to cause cancer). Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6	Destain the gel with two 15-min washes using distilled water.

To load a horizontal gel: *(continued)*

Step	Action
7	Visualize the DNA using a long-wave UV light box. **⚠ WARNING ULTRAVIOLET LIGHT HAZARD**. Exposure to ultraviolet radiation can cause blindness or permanent eye damage. To prevent eye injury, change the detector sensitivity from the ultraviolet to the visible range (520 nm) before beginning any detection or maintenance procedures. Always wear protective UV-absorbing glasses when looking into the detector. Turn off the lamp power before removing it from its fixture.

General Electrophoresis Conditions

Use the table below as a guide for running agarose gels.

Buffer	Chamber (cm)	DNA/lane (μg)	Volts/cm^2	Gel Thickness (mm)	Approx Run Time (hr)
TAE	12.5 x 20	1.0–5.0	4.5	3	4
	5.5 x 9	0.1–2.0	8.8	3	1
TBE	12.5 x 20	1.0–5.0	4.5	3	4
	5.5 x 9	0.1–2.0	8.8	3	1

Appendix A. How to Obtain Services and Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com,** then click the link for Services and Support.

At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

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