national diagnostics

# AquaPōr™ GTAC™

## Genetic Technology Analysis Certified™ Agaroses

AquaPōr LE GTAC
AquaPōr LM GTAC
AquaPōr ES GTAC
AquaPōr HR GTAC
AquaPōr 3:1 GTAC

## INTRODUCTION

All National Diagnostics' agaroses are made with complete control of the manufacturing process. Seaweed is selected by algologists only after visual inspection of the seaweed beds during scheduled dives. From the ocean to your laboratory, carefully monitored proprietary processing is paramount to supplying you with ultra pure agaroses suitable for your most valued biological samples.

The *GTAC*™ (*Genetic Technology Analysis Certified*™ ) designation indicates every procedure for which this material is certified has been tried and tested, assuring consistent and reliable results.

Proprietary processing with state-of-the-art manufacturing equipment maintains agarose polymer integrity, increases chain length and gel strength, and significantly improves gel handling characteristics. This assures crisp, clear gels with sharper bands and low background fluorescence. As an additional advantage, the higher density microcrystalline format makes weighing easier and more accurate, with less "fly-away" powder. Dissolution is rapid, and "boil over" is minimized.

| AquaPōr LE *all around performance* | | AquaPōr 3:1 *small fragments* | | AquaPōr LM *low melting point* | |
|---|---|---|---|---|---|
| Gel % | Size Range (bp) | Gel % | Size Range (bp) | Gel % | Size Range (bp) |
| 2.0 | 200 - 3,000 | 4 | 50 - 1000 | 4 | <500 |
| 1.75 | 250 - 4,000 | 3 | 100 - 1500 | 3 | 200 - 800 |
| 1.5 | 300 - 5,000 | 2 | 200 - 5000 | 2 | 300 - 3,000 |
| 1.0 | 400 - 12,000 | 1 | 400 - 10,000 | 1 | 500 - 20,000 |
| 0.75 | 1,000 - 23,000 | 0.75 | 1,000 - 20,000 | 0.75 | 600 - 25,000 |

| AquaPōr HR *highest resolution* | | AquaPōr ES *high strength for PFGE* | |
|---|---|---|---|
| Gel % | Size Range (bp) | Gel % | Size Range (bp) |
| 4.5 | 50 - 200 | 1.5 | 300 - 5,000 |
| 4.0 | 75 - 300 | 1.0 | 400 - 12,000 |
| 3.0 | 100 - 700 | 0.75 | 800 - 15,000 |
| 2.5 | 125 - 800 | 0.5 | 1,000 - 25,000 |
| 2.0 | 150 - 1000 | 0.3 | 5,000 - 50,000 |

*Gel percentage for optimal separation for fragment size ranges.*

## DISSOLVING AQUAPŌR AGAROSES

A. Add room temperature buffer to a flask that is 2.5 – 4 times the volume of gel solution. Add a teflon-coated stir bar.

B. Add AquaPōr powder while stirring vigorously so the agarose is dispersed uniformly. Stir for 2 minutes to hydrate the agarose.

C. Tare the flask and solution.

D. Place in a microwave oven and heat at 100% power using 20 – 60 second intervals. Swirl gently between intervals to resuspend the agarose.

E. Continue the cycle of heating and swirling until the agarose is completely dissolved (no visible particles are present).

F. Add distilled water to obtain the initial weight and mix.

G. Cool the solution to 50 – 60°C before pouring the gel.

* Chill the gel for 30 minutes prior to comb removal when using AquaPōr LM, HR, and low (<1%) concentration of AquaPōr LE and ES. This will complete gelation, increase gel strength, and enhance DNA resolution.

## OPTIMIZING DNA BAND RESOLUTION

Comb Selection:

- Use a thin (≤ 1 mm) comb with wide teeth for the sharpest, best resolved bands.
- Be certain the comb is cleaned scrupulously prior to use.

Gel Casting:

- Cast a gel that is ≤ 4 mm thick.
- A 20 cm long gel will be optimal for fine resolution of closely spaced DNA fragments.
- Chill the gel 30 minutes at 4°C prior to comb removal. This is particularly important for AquaPor LM, AquaPor HR and low concentration (<1%) AquaPor LE and ES. Chilling will complete gelling, increase gel strength, and optimize DNA band resolution.

Buffer Selection:

- Use 1X TBE for optimal resolution of DNA ≤ 12 kb when the DNA will not be recovered.
- Use 1X TAE for the best separation of DNA from 12 kb to 50 kb, or for DNA < 12 kb if the DNA will be recovered from the gel.
- Use 1X Tris-Acetate (TAE without EDTA) if the DNA will be used for in-gel enzymatic processing.
- Always overlay the gel with 3-4 mm buffer (just enough to cover the meniscus formed at the wells).

Sample Loading:

- Use a sample loading buffer containing Ficoll® type 400 polymer. Avoid glycerol containing loading buffers.
- Load sufficient sample such that the band(s) of interest contain 20 - 50 ng DNA, 100 ng is the maximum for the sharpest banding.

Electrophoretic Conditions:

- For DNA ≤ 12,000 bp use a voltage gradient of 5 - 6 V/cm where "cm" is the length between electrodes of the electrophoresis unit, NOT the length of the gel.
- For DNA 12,000 bp to 50,000 bp use a voltage gradient of 1 - 1.5 V/cm.
- Allow the DNA of interest to migrate 40-60% of the way through the gel.

## IN-GEL LIGATION AND TRANSFORMATION

INSERT PREPARATION

A. Cast an appropriate concentration AquaPōr LM gel using 40 mM Tris-Acetate buffer (1X TAE without EDTA).

B. Load sufficient sample to have 100-200 ng of insert DNA per band and electrophorese.

C. Stain for 10 minutes using freshly prepared ethidium bromide (0.5 to 1.0 µg/mL) in deionized water. Destain for 10 minutes in deionized water.

D. Excise the gel band(s) containing insert(s) and transfer to a sterile, preweighed microcentrifuge tube. Minimize exposure to UV (≥ 300 nm) radiation to less than 1 minute.

E. Determine gel volume by reweighing the tube plus gel slice. Assume 1 mg = 1 µL.

IN-GEL LIGATION

A. Melt AquaPōr LM gel slice by immersing tube in a 68°C bath.

B. Once melted, keep at 37°C until needed.

C. For a 25 µL ligation reaction, add the following, in order (Make additions to a microcentrifuge tube held at 37°C):

1. Distilled water sufficient for a 25 µL reaction volume.
2. 2.5 µL of 10X T4 DNA ligase buffer.
3. Sufficient vector for a 4:1 molar ratio of insert-to-vector.
4. T4 DNA ligase (1 Weiss unit).
5. Up to 12.5 µL of remelted AquāPor LM gel slice — Mix by resuspending components with pipette.
6. Ligate at the temperature recommended by the enzyme manufacturer.

TRANSFORMATION

A. Heat the ligation mixture at 68°C for 5 minutes to remelt.

B. Transfer ligation mixture to 37°C bath.

C. Place a sterile microcentrifuge tube containing 20 µL of sterile distilled water in the 37°C bath and add 5 µL of the ligation mixture.

D. Transfer 5-10 µL of the diluted ligation mixture to competent cells and follow the transformation protocol recommended for the cells you are using.

## IN-GEL PCR* (RE)AMPLIFICATION

A. Cast an appropriate concentration AquaPor LM or AquaPor HR gel using 40 mM Tris-Acetate (1X TAE without EDTA).

B. Load sufficient DNA to have 100-200 ng of DNA in the band(s) of interest, and electrophorese.

C. Stain for 10 minutes using freshly prepared ethidium bromide (0.5 to 1.0 µg/mL) in deionized water. Destain for 10 minutes in deionized water.

D. Excise the band(s) containing DNA and transfer to a sterile, preweighed microcentrifuge tube. Minimize exposure to UV (≥ 300 nm) radiation to less than 1 minute.

E. Determine gel volume by reweighing the tube plus gel slice. Assume 1 mg = 1 µL.

F. Melt AquaPor gel slice by immersing tube in a 68°C bath.

G. Once melted, keep at 37°C until needed.

H. Dilute a fraction of the melted gel slice such that 2 µL contains approximately 1 ng of DNA.

I. Transfer 2 µL of diluted gel mixture to a fresh PCR tube.

J. Add remaining components for PCR and perform amplification using conditions appropriate for the template and primers.

K. Remelt the reaction mixture at 65°C and electrophorese an aliquot on an AquaPor gel to determine the success of the reamplification.

## OPTIMIZING IN-GEL ENZYMATIC REACTIONS

- *Use 1X Tris-Acetate (TAE without EDTA) for the gel and electrophoresis buffer.* This will eliminate the addition of EDTA to subsequent In-Gel reactions, which would chelate $Mg^{+2}$, a necessary cofactor for most DNA processing enzymes. The absence of EDTA during electrophoresis will not affect DNA mobility or band resolution. Agarose gel electrophoresis buffers contain EDTA to chelate $Mg^{+2}$ and thereby inhibit endogenous nucleases. All AquaPor agaroses are certified DNase and RNase free and therefore, electrophoresis may be performed confidently in the absence of EDTA when the DNA will be used for subsequent in-gel enzymatic processing.

- *Do not add ethidium bromide to the gel prior to electrophoresis.* DNA electrophoresis in the presence of ethidium bromide can reduce the biological activity of the electrophoresed DNA. Briefly stain the gel after electrophoresis in freshly prepared 0.5-1.0 µg/mL ethidium bromide, and destain in deionized water.

- *Load sufficient DNA to have 50-200 ng of DNA in the band(s) of interest.* Use a 1 mm thick comb to keep the DNA bands sharp and minimize the size of the gel slice.

- *Do not exceed 68°C when remelting the gel slice.* Small DNA fragments may denature above 68°C.

- *Use a UV light source (> 300 nm) and keep UV exposure to less than 1 minute.* Prolonged UV exposure will cause photonicking and photodimerization, compromising the biological activity of the DNA.

# AquaPōr™ Agaroses

| | | |
|---|---|---|
| AquaPōr LE GTAC™<br>Order No. EC-202 | 25 grams<br>100 grams<br>500 grams | **Multi-Purpose Low EEO** |
| AquaPōr ES GTAC<br>Order No. EC-203 | 25 grams<br>100 grams | **High Strength** |
| AquaPōr LM GTAC<br>Order No. EC-204 | 25 grams<br>100 grams | **Low Melting** |
| AquaPōr HR GTAC<br>Order No. EC-205 | 25 grams<br>100 grams | **High Resolving** |
| AquaPōr 3:1 GTAC<br>Order No. EC-206 | 25 grams<br>100 grams | **Small Fragments** |

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