



AquaPör™ GTAC™

Genetic Technology Analysis Certified™ Agaroses

AquaPör LE GTAC

AquaPör LM GTAC

AquaPör ES GTAC

AquaPör HR GTAC

AquaPör 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 the latest in 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.

AquaPör LE <i>all around performance</i>		AquaPör 3:1 <i>small fragments</i>		AquaPör LM <i>low melting point</i>	
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

AquaPör HR <i>highest resolution</i>		AquaPör ES <i>high strength for PFGE</i>	
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 AQUAPÖR AGAROSSES

- Add room temperature buffer to a flask that is 2.5 – 4 times the volume of gel solution. Add a teflon-coated stir bar.
- Add AquaPör powder while stirring vigorously so the agarose is dispersed uniformly. Stir for 2 minutes to hydrate the agarose.
- Tare the flask and solution.
- Place in a microwave oven and heat at 100% power using 20 – 60 second intervals. Swirl gently between intervals to resuspend the agarose.
- Continue the cycle of heating and swirling until the agarose is completely dissolved (no visible particles are present).
- Add distilled water to obtain the initial weight and mix.
- Cool the solution to 50 – 60°C before pouring the gel.

* Chill the gel for 30 minutes prior to comb removal when using AquaPör LM, HR, and low (<1%) concentration of AquaPör 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 AquaPör LM, AquaPör HR and low concentration (<1%) AquaPör 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 Ficol[®] 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 $\leq 12,000$ 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

- Cast an appropriate concentration AquaPör LM gel using 40 mM tris-acetate buffer (1X TAE without EDTA).
- Load sufficient sample to have 100-200 ng of insert DNA per band and electrophorese.
- Stain for 10 minutes using freshly prepared ethidium bromide (0.5 to 1.0 $\mu\text{g}/\text{mL}$) in deionized water. Destain for 10 minutes in deionized water.
- 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.
- Determine gel volume by reweighing the tube plus gel slice. Assume 1 mg = 1 μL .

IN-GEL LIGATION

- Melt AquaPör LM gel slice by immersing tube in a 68°C bath.
- Once melted, keep at 37°C until needed.
- For a 25 μL ligation reaction, add the following, in order (Make additions to a microcentrifuge tube held at 37°C):
 - Distilled water sufficient for a 25 μL reaction volume.
 - 2.5 μL of 10X T4 DNA ligase buffer.
 - Sufficient vector for a 4:1 molar ratio of insert to vector.
 - T4 DNA ligase (1 Weiss unit).
 - Up to 12.5 μL of remelted AquaPör LM gel slice—mix by resuspending components with pipette.
 - Ligate at the temperature recommended by the enzyme manufacturer.

TRANSFORMATION

- Heat the ligation mixture at 68°C for 5 minutes to remelt.
- Transfer ligation mixture to 37°C bath.
- 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.
- 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

- Cast an appropriate concentration of AquaPör LM or AquaPör HR gel using 40 mM tris-acetate (1X TAE without EDTA).
- Load sufficient DNA to have 100-200 ng of DNA in the band(s) of interest, and electrophorese.
- Stain for 10 minutes using freshly prepared ethidium bromide (0.5 to 1.0 $\mu\text{g}/\text{mL}$) in deionized water. Destain for 10 minutes in deionized water.
- 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.
- Determine gel volume by reweighing the tube plus gel slice. Assume 1 mg = 1 μL .
- Melt AquaPör gel slice by immersing tube in a 68°C bath.
- Once melted, keep at 37°C until needed.
- Dilute a fraction of the melted gel slice such that 2 μL contains approximately 1 ng of DNA.
- Transfer 2 μL of diluted gel mixture to a fresh PCR tube.
- Add remaining components for PCR and perform amplification using conditions appropriate for the template and primers.
- Remelt the reaction mixture at 65°C and electrophorese an aliquot on an AquaPör 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 AquaPör 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 $\mu\text{g}/\text{mL}$ 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 photoniccking and photodimerization, compromising the biological activity of the DNA.

AquaPör™ Agaroses

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

For Additional Information And Order Placement:

USA: Toll Free: (800) 526-3867 Georgia: (404) 699-2121 Fax: (404) 699-2077 e-mail: info@nationaldiagnostics.com	National Diagnostics, Inc. 305 Patton Drive Atlanta, GA 30336
EUROPE: Phone: 0115 982 1111 Fax: 0115 982 5275 e-mail: slsinfo@scientific-labs.com	Scientific Laboratory Supplies, Ltd. Wilford Industrial Estate Ruddington Lane Nottingham, NG11 7EP