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Lambda DNA ladders in InCert™ agarose gel plugs size standards for megabase DNA

Certificate of performance

Introduction

The lambda phage cl857S7 is the source of DNA oligomers useful for markers ranging from 48.5 kb up to at least 873 kb in size, each rung differing by 48.5 kb. These standards are prepared in gel plugs of low gelling/melting temperature InCert® agarose specifically designed for preparation of chromosomal DNA in gel plugs by the Schwartz and Cantor method.[1] Like other Genetic Technology Grade™ products, these standards are tested and certified for a specific end-use application. Each lot is certified to yield excellent resolution with low background, sharp bands, and consistent marker sizes after pulsed field gel (PFG) electrophoresis.[2] These standards are useful for finer size estimates of DNA 873 kb.[3-5]

Contents

5 plugs (2 x 5 x 10 mm)
Each plug yields approximately 4-6 lanes as determined on the Pulsaphor® system.

Quality assurance test results

Typical Lot data

1. 10 ± 1 μg DNA/plug
2. Presence of at least 18 bands verified by PFGE and ethidium bromide staining
3. Low background and sharp bands
4. Relative mobility of lambda DNA ladder (cl857S7) confirmed, compared to *S. cerevisiae* chromosomal DNA (YPH80)

Quality assurance test

The lambda DNA ladder standards in 0.75% Incert™ agarose Gel Plugs are electrophoresed in a gel under conditions listed below and stained with ethidium bromide. The mobilities of rungs of the lambda ladder are compared to that of *S. cerevisiae* chromosomal DNA prepared in InCert™ Agarose Gel Plugs and run on the same gel. The amount of DNA per plug has been determined by A_{260}/A_{280} of phage DNA entrapped in each plug.

Running Conditions for Quality Assurance Test:

Electrophoresis equipment:	Pulsaphor® system
Running gel:	1% SeaKem® GTG™ agarose gel (20 x 20 cm)
Running buffer:	100 mM Tris, 100 mM Boric acid 0.2 mM EDTA, pH 8.0
Temperature:	12°C
Voltage:	10 V/cm, 330 V (33 x 33 cm gel box)
Pulse time:	100 seconds
Length of run:	40 hours

Storage and handling conditions

Storage conditions: refrigerate at 2ºC-8ºC; do not freeze.
***Storage buffer contains*:**
50 mM ethylenediaminetetraacetic acid
10 mM [Tris (hydroxymethyl) aminoethane], pH 8.0
5 μg/ml proteinase K
0.01% N-lauroyl sarcosine
Note: Care should be taken in handling storage buffer and plugs as proteinase K is a powerful protease.

Handling standards-use sterile technique for removing plugs from bottle.
-Decant the storage buffer until the top of the plugs are visible. Use a sterile, flame-sealed, bent-tipped pasteur pipette to remove the gel plugs from the bottle.
Store gel plugs removed from the bottle in another sterile tube with fresh filter-sterilized, storage buffer.

Loading the gel plug slice onto the running gel
-We routinely load ¼ of the gel plug for electrophoresis on the Pulsaphor® system and slice the gel plug using a glass cover slip. If the running buffer is 1X TBE, there is no need to dialyze the gel slice. However, if the running buffer is 1X TAE or lower conductivity buffer, dialyze the gel slice by two 30 minutes washes in that particular buffer.

- There are two ways to load gel slices.

1- Gently push the gel slice into a well with an alcohol sterilized glass rod, keeping the slice intact, until it touches the bottom of the well. Avoid trapping air bubbles. Overlay the gel slice with liquid agarose of the same type and concentration as the running gel.

2- Apply the gel slices to the teeth of a comb and place comb in chamber with the gel slices facing the direction of migration.

Then pour the agarose running gel; remove comb when the gel is set; add electrophoresis buffer and electrophorese as usual.[6]

NOTE: Lambda DNA ladders do not withstand melting at 70°C. Therefore, liquid loading is not recommended.

References

1. Schwartz, D.C. and Cantor, C.R. (1984) ***Cell 37***: 67-75.
2. Schwartz, D.C., Saffran, W., Welsh, J., Haas, R., Goldenberg, N., and Cantor, C., (1983) ***Cold Spring Harbor Symposia on Quant. Biol. XLVII:*** 189-195.
3. Carle, G.F. and Olson, M.V. (1984) ***Nucl. Acids Res. 12***: 5647-5664
4. Bernards, A., Kooter, J.M., Michels, P.A.M., Moberts, R.M.P. and Borst, P., ***Gene, 42***: 313-322
5. Southern, E.M., Anand, R., Brown, W.R.A., and Fletcher, D.S., (1987) ***Nucl. Acids Res. 15***: 5925-5943
6. Birren, B., Lai, E., Clark, S.M., Hood, L., and Simon,M.I. (1988) ***Nucl. Acids Res. 16***: 7563-7582

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Not for use in diagnostic procedures.

Patent position
Lonza does not warrant against infringements of other patents of third parties by reason of any uses made of the product in combination with other material or in the operation of any process, and purchasers assume all risks of patent infringement by reason of any such use, combination, or operation.

Product safety
Agarose, DNA, and other chemicals in this product are not hazardous chemicals within OSHA Hazard Communication Standard. For details, see Material Safety Data Sheet (M.S.D.S.) call (800) 638-8174 or go to our website **www.lonza.com.** Emergency number after hours, call collect +1 (202) 483-7616.

Warranty
Because of the numerous factors affecting results, Lonza's DNA standards in agarose gel plugs are sold with the understanding that purchasers will make their own tests to determine the suitability of these plugs for their particular purposes. The use suggested by Lonza is presented only to assist our customers in exploring possible applications for this product. All information and data presented are believed to be accurate and reliable but are presented without the assumption of any liability by Lonza.

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Ordering information:

Catalog No.	Size
50401	1 bottle

5 plugs (2 x5 x 10 mm each)

Related products
InCert™ agarose
SeaKem® GTG™ agarose

For more information contact Lonza scientific support at (800) 521-0390 or visit our website at www.lonza.com.