BIOTECH SUPPORT GROUP

ProPrep™ Omni BAC 200

Large culture BAC DNA-purification with ProCipitate™

Product	Size	Item No.
ProPrep™ Omni BAC 200	20 to 200ml cultures	PPO-200

ProPrep™ Omni BAC 200 is a complete purification system based upon the proprietary reagent, ProCipitate™[1]. ProCipitate™ has been demonstrated to provide high quality DNA suitable for automated fluorescent sequencing of small to large insert DNA[2,3,4,5,6,7,8].

The ProPrep™ strategy is to remove only the contaminants and leave DNA alone. This minimizes shearing, improves yield and makes ProPrep™ Omni BAC 200 ideal for template DNA preparation and transfection of plasmids, cosmids and BACs.

The ProPrep™ Omni BAC 200 system starts with 20ml up to to 200 ml cultures, and then utilizes ProCipitate™ in a modified alkaline lysis protocol.

BENEFITS

- High Yield of 30 - 50 μg BAC DNA
- Scaleable - 20 ⇒ 200 ml starting culture
- Economical
- Purity suitable for sequencing & transfection
- Plasmids ⇒ BACS, 2 ⇒ 300 kb inserts

MATERIALS AND SCOPE OF SUPPLY

Items Required	Quantity	ProPrep™ Omni	Storage
ProCipitate™	5 ml	Supplied	4°C (best if used before date on label)
0.45 μm cellulose acetate vacuum filter	2	Supplied	---
TE1 Resuspension Buffer	10 ml	Supplied	Room Temp. (or 4°C after RNase addition)
RNase Cocktail	1 ml	Supplied	-20°C
AL2 Lysis Buffer If precipitate forms, solubilize by placing bottle in warm water	10 ml	Supplied	Room Temp.
NB3 Neutralization Buffer	10 ml	Supplied	Room Temp.
Isopropanol		Not Supplied	---
70% Ethanol		Not Supplied	---
Resolubilization Buffer		Not Supplied	---
Wide Bore Pipette Tips		Not Supplied	---

1 Deer Park Drive, Suite M, Monmouth JCT, NJ 08852, USA • (P) 732-274-2866 • (F) 732-274-2899
www.biotechsupportgroup.com

BIOTECH SUPPORT GROUP

PROTOCOL

Proper mixing is imperative to insure consistent results. For optimum performance follow mixing instructions shown.

1. Grow 200 ml overnight culture in LB broth (for BACs 20 μg/ml chloramphenicol) to early stationary phase, a concentration range from $2 \rightarrow 4x10^9$; corresponds to OD_{600} (1:10 dilution) = 0.2→0.4. TB and other enriched broths are not recommended for this protocol.

2. Concentrate cells at [2,500 x g] for 10 minutes.

3. Decant and discard supernatant. Add 10 ml TE1 and 800 μl RNase cocktail, vortex to resuspend the cells and transfer to a 50 ml clean centrifuge tube.

4. Add 10 ml of AL2, mix by slow inversion for 1 minute and leave at room temperature for 2 minutes.

5. Add 10 ml of NB3 and mix by slow inversion for 1 minute and leave at room temperature for 3 minutes.

6. Shake ProCipitate™ well to completely resuspend. Using a wide bore pipette tip, add 5 ml of ProCipitate™. VERY IMPORTANT - Mix by pipetting up and down with a wide bore pipette tip, 5 ml ones are preferable. For 5 ml tips, repeat 5 times, for 1 ml tips, repeat 20 times.
7. Apply vacuum source to filter provided. Transfer all of the contents to the 0.45 μm cellulose acetate vacuum filter. Continue to apply vacuum until the sample has been drawn through. For scale down version of this protocol (i.e., 20 ml starting culture), additional filters are supplied as accessories.

8. Transfer the filtrate into a clean tube. Add 18 ml of 100% Isopropanol (room temperature) to the sample, shake briefly to mix and leave at room temperature for 20 minutes.

9. Centrifuge at [2,000 x g] for 15 minutes to pellet the DNA.

10. Decant and discard supernatant.

11. Wash pellets with 10 ml of 70% ethanol (room temperature) by shaking the tube gently from side to side to resuspend the pellet, then centrifuge at [2,000 x g] for 10 minutes.

12. Decant and discard the supernatant and air or vacuum-assist dry the pellet until no visible drops remain.

1 Deer Park Drive, Suite M, Monmouth JCT, NJ 08852, USA • (P) 732-274-2866 • (F) 732-274-2899
www.biotechsupportgroup.com

13. Resolubilize in 50-100 µl of TE, DI water, or other suitable buffer, cap tightly and incubate for 30 - 60 minutes at 65°C to assist resolubilization. Shake once or twice during this period to assist resolubilization.

PROTOCOL, cont.

- This protocol can be scaled down linearly & adapted to cultures as small as 20ml.
- For cultures 200 to 1000ml, use ProPrep™ Omni BAC 200.
- Replacement 0.45 µm cellulose acetate vacuum filters are available for purchase (Catalog # FV08, package of 5).

For mini-prep and high throughput 96 well formats, try ***ProPrep™ Mini BAC*** or ***ProPrep™ Mini BAC 96 well***.

REFERENCES

1. U.S. Patent Numbers 5,294,681, 5,453,493 and other patents pending.
2. Huang, G.M., et al, *A High-Throughput Plasmid DNA Preparation Method*, Analytical Biochem, 223:35-48, 1994.
3. Kelley, J.M., et al, *High-Throughput Direct End Sequencing of BAC Clones*, Nucleic Acids Reseach, Vol. 27, No. 6: 1539-1546, 1999.
4. http://www.hgmp.mrc.ac.uk/ISO9000/BIOLOGY/LIBRARIES/pig_BAC/procipitate.shtml
5. PE Biosystems User Bulletin. Subject: Sequencing Large DNA Templates.
6. Reddy, O.U.K., et al, *New Dinucleotide and Trinucleotide Microsatellite Marker Resources for Cotton Genome Research*, Journal of Cotton Science, 5:103-113 (2001).
7. Klein, R.K., et al, *High Throughput BAC DNA Isolation for Physical Map Construction of Sorghum*, Plant Molecular Biology Reporter, Kluwer Academic Publishers, 1998.
8. Bruce, D.C., et al, *BAC Library End Sequencing in Support of Whole Genome Assemblies*, poster DOE Joint Genome Institute and Center for Human Genome Studies, Los Alamos National Laboratory.

CONTACT US

We welcome your questions and comments regarding our products.

Call 732-274-2866, 800-935-0628 (North America) Mon – Fri 9am-6pm EST.

Fax 732-274-2899

Email **sales@biotechsupportgroup.com**

Mail 1 Deer Park Drive, Suite M, Monmouth JCT, NJ 08852, USA