

Dharmacon™ cDNA Clones

Product Description

Clones are provided as *Escherichia coli* cultures in LB broth with 8% glycerol, an inert growth indicator, and the appropriate antibiotic at the concentration indicated in Table 1.

Shipping and Storage:

Individual clones are shipped at room temperature and may be stored for up to one week at +4 °C. Orders of greater than 50 individual clones will be arrayed in a 96 well format. Plates are shipped on dry ice and should be stored at -80 °C. Clones may be stored indefinitely at -80 °C.

Clone Verification:

All DNA sequences and annotations have been submitted to GenBank by the supplier at the time of library creation (For example, the IMAGE Consortium), but have not been independently verified by Dharmacon. Information about a particular clone can be found by entering the Clone ID number or accession number into search box at the top right of the webpage at (dharmacon.com). Further verification can be performed by using BLAST or other bioinformatic tools (blast.ncbi.nlm.nih.gov/Blast.cgi). If you have difficulty finding your clone please contact Technical Support (ts.dharmacon@horizondiscovery.com, 800 235 9880) for help.

Protocol

Sequence Verifying Individual cDNA Identity:

To ensure you are working with a clone is derived from a single isolate, streak out and isolate at least 3 individual colonies for sequencing. The sequencing primers listed under the details tab for each clone. In cases of incorrect clone identification please contact Technical Support (ts.dharmacon@horizondiscovery.com, 800 235 9880) with your order information and sequencing data and we will supply the correct clone if possible.

Making A Stock Culture:

Once the clone has been streak isolated and the identity of the clone has been confirmed, we recommend making a stock of the pure culture. Grow the pure culture in LB broth with the appropriate antibiotic Transfer 920 µL of culture into a polypropylene tube and add 80 µL sterile glycerol to make an 8% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at -80 °C.

Plate Replication Protocol

Prepare Target Plates:

- Dispense ~ 160 µL of sterile LB medium into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare Source Plates:

- Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
- Thaw the source plate.

Replicate

- Gently place a disposable replicator into the thawed source plate and gently move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the well.
- Gently remove the replicator from the source plate and carefully place the replicator into the target plate.
- Gently move the replicator back and forth in the target plate to transfer cells.
- Discard the replicator.
- Place the lids back on the source plates and target plates.

Table 1. Cap color code

Color	Antibiotic	Concentration
Red	Carbenicillin*	100 µg/mL
Black	Chloramphenicol	25 µg/mL
Green	Kanamycin	25 µg/mL

*Ampicillin can also be used at the same concentration

Table 2. Materials for plate replication

Item	Vendor	Cat #
2x LB-Lennox Broth (low salt)	Fisher Scientific™	BP1427500
Glycerol	Fisher Scientific™	BP2291
Carbenicillin or Ampicillin	Fisher Scientific™	BP2648-5 or BP1760-5
Chloramphenicol	Fisher Scientific™	BP904-100
Kanamycin	Fisher Scientific™	BP906-5
96-well microplates	Nunc™	12-565-363
Aluminum seals	Nunc™	12-565-475
Disposable replicators	Fisher Scientific™	NC9584102

- Seal the source plates, being mindful to avoid cross contamination.
- Repeat this process until all plates have been replicated.
- Return the source plates to the -80 °C freezer.
- Place the inoculated target plates in a 37 °C incubator. Incubate the plates for 12-24 hours.

FAQS/Troubleshooting

For additional information, please contact Technical Support (ts.dharmacon@horizondiscovery.com, 800 235 9880).

Questions	Answers
Where can I find a complete vector map of my cDNA clone?	We were not provided with complete maps for the clones in this collection, but the information available online can be used to create a map. You can find the details for your clone by clicking on the [+] next to your clone's description. The "Details" tab includes a hyperlink to the plasmid sequence as well as an indication of the 5' and 3' cloning restriction sites. The "Sequence" tab includes a link the clone's insert sequence.
What is the appropriate antibiotic to use with my cDNA clone?	Clones are provided as <i>E. coli</i> cultures in LB broth with 8% glycerol, an inert growth indicator, and the appropriate antibiotic at the concentration indicated either from our website or using the tube cap color as an identifier. The cap color codes are below: Red - Ampicillin (100µg/ml) Black - Chloramphenicol (25µg/ml) Green - Kanamycin (25µg/ml)
Why is my bacterial glycerol stock yellow?	Some of our <i>E. coli</i> cultures are grown with an inert growth indicator, phenol red. This indicator is red at pH 8.2 and above and begins to turn orange and then yellow as the pH drops. At pH 6.8 the indicator is yellow. As the <i>E. coli</i> are growing they give off CO ₂ which decreases the pH of the broth and changes the color of the indicator from red to yellow.
What would you suggest I do if my cDNA clone is not growing?	First ensure that you are using the correct antibiotic and concentration for your clone. You can find this information as described in the Clone Details section of this document. Additional strategies that may be used to spur growth include: <ul style="list-style-type: none"> • Try inoculating from thawed source tube rather than frozen. • Gently mix the source tube by inversion (with the lid on) to ensure the cells are not settled in the bottom before the inoculum is taken. • Try using broth culture instead of plate culture to jumpstart growth. • Use shaking during growth of the broth culture. • Try increasing the inoculum (i.e. 10ul inoculum into 2ml broth). • Try spinning the tube to pellet the cells and take a portion of the pellet to inoculate a broth culture.

Good Faith Agreement / License Agreements

For questions regarding license agreements please visit: dharmacon.gelifsciences.com/uploadedFiles/dharmacon-product-terms-and-conditions.pdf and/or contact Technical Support (ts.dharmacon@horizondiscovery.com, 800 235 9880).

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+1 800 235 9880; +1 303 604 9499 if you have any questions.