Big Blue Cell Lines

INSTRUCTION MANUAL

Catalog #726000 (Big Blue Rat Cell Line)
Revision A

For In Vitro Use Only
726000-12

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BIG BLUE CELL LINES

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Big Blue Cell Lines

Catalog #726000

MATERIALS PROVIDED

Materials provided[a]	Quantity
Big Blue rat cell line	1-ml cryovial (~1 × 10^6 cells)

[a] Immediately on receipt, store the cryovial of cells in liquid nitrogen. Protective gloves, clothing, and a face mask or goggles should be worn when transferring the cryovial to and from liquid nitrogen.

STORAGE CONDITIONS

Store immediately in liquid nitrogen.

ADDITIONAL MATERIALS REQUIRED

G418 Sulfate [Stratagene Catalog #200399 (1 g)]
Liquid nitrogen storage tank
Growth medium[§]
Freezing medium[§]
Phosphate-buffered saline (PBS)[§]
Plasticware
Incubator (5–7% CO_2, 37°C)
Controlled freezing container [e.g., the StrataCooler Cryo preservation module (Stratagene Catalog #400005), or the equivalent)
Tabletop centrifuge
Rubber policeman
Trypsin–EDTA (0.05% trypsin and 0.53 mM tetrasodium ethylenediaminetetraacetic acid)
Big Blue Transgenic Rodent Mutagenesis Assay System Instruction Manual
RecoverEase DNA isolation kit [Stratagene Catalog #720202 (30 preparations), Catalog #720203 (10 preparations)]

[§]See Preparation of Media and Reagents.

Revision A

INTRODUCTION

The Big Blue rat cell line is useful for *in vitro* mutagenesis studies, and is a part of the Big Blue transgenic rodent mutagenesis assay system, which is used to assess the genetic toxicity of test compounds.[1–4]

The Big Blue rat cell line is derived from a Rat2 embryonic fibroblast cell line transfected with the Big Blue λLIZ shuttle vector and the pSV2NEO plasmid.[5, 6] The Big Blue λLIZ shuttle vector (Figure 1) is 45 kb in size and contains the *lacI* and α*lacZ* genes. Each cell contains 50–70 copies of the λLIZ shuttle vector integrated into the genome at two sites. The pSV2NEO plasmid provides resistance to antibiotic G418. The Big Blue rat cell line is a thymidine kinase mutant, and the chromosome number is polyploid.

The *lacI* gene in the Big Blue λLIZ shuttle vector is the target for mutagenesis. After the cells are treated with a test compound, *in vitro* packaging rapidly and efficiently recovers the λLIZ shuttle vector from the genomic DNA of the cell. Bacterial detection systems screen for mutations in the target gene. The target gene can be recovered from mutant plaques for sequence analysis. In addition to short-term *in vitro* mutagenesis studies, these cell lines are useful for various toxicological applications, studies of the effects of oncogenes and tumor suppressor genes on mutation frequencies, and studies of DNA repair.

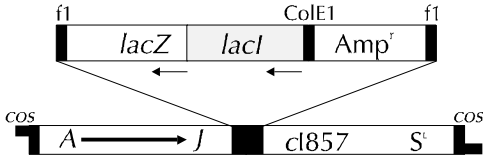

Figure 1 The Big Blue λLIZ shuttle vector.

CELL CULTURE PROTOCOLS

Starting the Cells in Culture

1. Prepare the growth medium.[§] Place 10 ml of growth medium in a 15-ml conical tube.

2. Thaw the frozen cryovial of cells within 40–60 seconds by rapid agitation in a 37°C water bath. Remove the cryovial from the water and immediately immerse the cryovial in 70% (v/v) ethanol at room temperature.

   Note *All operations from this point should be carried out using sterile technique.*

3. Transfer the cell suspension to the conical tube containing the growth medium.

4. Centrifuge the cells at 200 × *g* for 5 minutes at room temperature. Remove the growth medium from the conical tube by aspiration.

5. Place 10 ml of growth medium in a 75-cm^2 tissue culture flask. Resuspend the cells in the conical tube in 5 ml of growth medium. Transfer the cell suspension to the tissue culture flask.

   Note *Add 200 µg/ml of G418 Sulfate to the growth medium for the Big Blue rat cell line.*

6. Place the cells in a 37°C incubator with 5–7% CO_2.

[§] See *Preparation of Media and Reagents.*

Passaging the Cells

Note *Split the cells when the monolayer of cells is ~80–90% confluent or approximately every 3–4 days.*

1. Wash the cells with PBS. Remove the PBS by aspiration.

2. Add 1.5 ml of trypsin–EDTA to each 75-cm^2 tissue culture flask. Incubate the cells at room temperature for ~2–5 minutes. Tap the flask to release the adherent cells.

   Note *Incubate the cells with trypsin–EDTA for the minimum time necessary for the adherent cells to release from the flask. Overexposure to trypsin can kill the cells.*

3. Dilute the cells with growth medium to a final volume of 5–10 ml. The serum in the medium inactivates the trypsin. Transfer the cells to the desired number of 75-cm^2 or 175-cm^2 tissue culture flasks. Depending on the desired use for the cells, use an inoculum of 1×10^3 to 1×10^6 cells. Add growth medium to the tissue culture flasks to a final volume of 15 ml for each 75-cm^2 flask or 30 ml for each 175-cm^2 flask.

4. Place the cells in a 37°C incubator with 5–7% CO_2.

Freezing the Cells for Long-Term Storage

Note *Maintain freezer stocks of early-passaged cells to ensure long-term stability of the cell line.*

1. Wash the cells with PBS. Remove the PBS by aspiration.

2. Add 1.5 ml of trypsin–EDTA to each 75-cm^2 tissue culture flask. Incubate the cells at room temperature for ~2–5 minutes. Tap the flask to release the adherent cells.

   Note *Incubate the cells with trypsin–EDTA for the minimum time necessary for the adherent cells to release from the flask. Overexposure to trypsin can kill the cells.*

3. Dilute the cells with 8.5 ml of growth medium. The serum in the medium inactivates the trypsin.

4. Transfer the cell suspension to a 15-ml conical tube. Centrifuge the cells at $200 \times g$ for 5 minutes at room temperature. Remove the growth medium from the tube by aspiration.

5. Resuspend the cells in 1–2 ml of freezing medium.

6. Transfer the cells to cryovials. Place the cryovials in a chilled controlled freezing container. Incubate the cryovials at –80°C overnight.

7. The following day, transfer the cryovials to liquid nitrogen for long-term storage, taking the proper safety precautions for working with liquid nitrogen. If properly stored, the cells should remain stable for >1 year.

Treating the Cells with a Test Compound

Note *Theoretically, 150,000 cultured cells yields ~1 µg of genomic DNA. The Big Blue rat cell line achieves rescue efficiencies of ~50,000–100,000 pfu/µg of genomic DNA.*

1. Treat the cells with the test compound of interest when the monolayer of cells is ~30–40% confluent or is at the confluence stated in an established protocol for the test compound.[5, 6]

2. After treatment with the test compound and the desired expression time, isolate the DNA for mutagenesis studies. To isolate the DNA, remove the growth medium and wash the cells with PBS.

3. Harvest 1×10^7 to 2×10^7 cells in ~5 ml of PBS by scraping the surface of the tissue culture flasks with a rubber policeman.

4. Transfer the cell suspension to a 15-ml conical tube. Centrifuge the cells at 200 × *g* for 5 minutes at room temperature. Remove the supernatant from the tube by aspiration.

   Note *At this point, the pellet can be flash frozen in liquid nitrogen and stored at –80°C until DNA isolation is performed.*

5. Isolate the DNA from the cells. Proceed with step 3 of *Tissue Culture* in the *RecoverEase DNA Isolation Kit Instruction Manual*.

TROUBLESHOOTING

Observation	Suggestion(s)
The cells do not survive following resuspension in growth medium after storage in liquid nitrogen	Ensure that the cells are stored properly. Store the cryovials of cells in liquid nitrogen immediately on receipt.
	Ensure that the cells are thawed properly. Thaw the cells quickly and immediately dilute the cells in the growth medium.
The cells do not survive passaging	Ensure that the cells are passaged using the recommended conditions. When passaging, inoculate a fresh tissue culture flask with 1×10^3 to 1×10^6 cells.
	Split the cells when the monolayer of cells is at most 90% confluent to avoid starving the cells for growth medium.
	The cells may have been exposed to trypsin–EDTA for too long. Incubate the cells with trypsin–EDTA for the minimum time necessary for releasing the cells from the flask.
Low rescue efficiencies	Errors in preparing or packaging genomic DNA may cause low rescue efficiencies. Follow the instructions carefully in the *Big Blue Transgenic Rodent Mutagenesis Assay System Instruction Manual*.

PREPARATION OF MEDIA AND REAGENTS

Freezing Medium

9 ml of fetal bovine serum
1 ml of dimethylsulfoxide (DMSO)
Filter sterilize

Growth Medium

500 ml of Dulbecco's Modified Eagle Medium (DMEM) (high glucose, with L-glutamine, without sodium pyruvate)
5 ml of 200 mM L-glutamine
5 ml of penicillin (5000 U/ml)–streptomycin (5000 μg/ml) mixture
50 ml of fetal bovine serum, heat inactivated

PBS

137 mM NaCl
2.6 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4
Adjust the pH to 7.4 with HCl

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4. Rogers, B. J., Provost, G. S., Young, R. R., Putman, D. L. and Short, J. M. (1995) *Mutat Res* 327(1-2):57-66.
5. Manjanatha, M. G., Chen, J. B., Harris, A. J., Shaddock, J. G., Jr. and Casciano, D. A. (1996) *Environ Mol Mutagen* 27:44.
6. Wyborski, D. L., Malkhosyan, S., Moores, J., Perucho, M. and Short, J. M. (1995) *Mutat Res* 334(2):161-5.

ENDNOTES

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The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

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Big Blue Cell Lines

Catalog #726000

QUICK-REFERENCE PROTOCOL

Starting the Cells in Culture

- Place 10 ml of growth medium in a 15-ml conical tube
- Thaw the frozen cells quickly in a 37°C water bath
- Transfer the cell suspension to the conical tube containing the growth medium
- Centrifuge the cells at 200 × *g* for 5 minutes at room temperature and remove the growth medium by aspiration
- Place 10 ml of growth medium in a 75-cm^2 tissue culture flask, resuspend the cells in the conical tube in 5 ml of growth medium, and transfer the cell suspension to the tissue culture flask

  Note *Add 200 µg/ml of G418 Sulfate to the growth medium for the Big Blue rat cell line*

- Place the cells in a 37°C incubator with 5–7% CO_2

Passaging the Cells

- Wash the cells with PBS
- Add 1.5 ml of trypsin–EDTA to each 75-cm^2 tissue culture flask, incubate the cells at room temperature for ~2–5 minutes, and tap the flask to release the adherent cells
- Dilute the cells with the growth medium and transfer the cells to the desired number of tissue culture flasks
- Place the cells in a 37°C incubator with 5–7% CO_2

Freezing the Cells for Long-Term Storage

- Wash the cells with PBS
- Add 1.5 ml of trypsin–EDTA mixture to each 75-cm^2 tissue culture flask containing cells, incubate the cells at room temperature for ~2–5 minutes, and tap the flask to release the adherent cells
- Dilute the cells with 8.5 ml of growth medium
- Transfer the cell suspension to a 15-ml conical tube, centrifuge at 200 × *g* for 5 minutes at room temperature, and remove the growth medium by aspiration
- Resuspend the cells in 1–2 ml of freezing medium
- Transfer the cells to cryovials, place the cryovials in a chilled controlled freezing container, and incubate the cryovials at –80°C overnight
- The following day, transfer the cryovials to liquid nitrogen for long-term storage

Treating the Cells with a Test Compound

- Treat the cells with the test compound when the monolayer is ~30–40% confluent or is at the confluence stated in the protocol for the test compound
- To isolate DNA for mutagenesis studies after treatment with the test compound, remove the growth medium and wash the cells with PBS
- Harvest 1×10^7 to 2×10^7 cells in ~5 ml of PBS by scraping the surface of the tissue culture flasks with a rubber policeman
- Transfer the cell suspension to a 15-ml conical tube, centrifuge at 200 × *g* for 5 minutes at room temperature, and remove the supernatant by aspiration
- If DNA isolation will be performed immediately, resuspend the cells in 5 ml of ice-cold lysis buffer and proceed with step 3 of *Tissue Culture* in the *RecoverEase DNA Isolation Kit Instruction Manual*
- If DNA isolation will not be performed immediately, flash freeze the pellet by immersing the 15-ml conical tube containing the cells in liquid nitrogen and store the frozen cells at –80°C until DNA isolation is performed