

BNL CL.2 Cells | 305177**General information****Description**

BNL CL.2, a mouse liver cell line originally derived from BALB/c embryonic liver cells, plays a significant role in the study of cellular biology and molecular mechanisms, especially regarding the cell cycle and its regulation. Researchers have extensively used BNL CL.2 to characterize cyclin-dependent kinase (CDK) protein complexes and investigate the alterations in these complexes following both chemical and viral transformation. This line serves as a progenitor for various transformed cell lines such as BNL 1ME A.7R.1, BNL 1NG A.2, and BNL SV A.8, all of which originate from BNL CL.2 and have proven essential for studying CDK alterations post-transformation.

BNL CL.2 is distinguished by its non-tumorigenic nature when tested in immunosuppressed mice, and its inability to grow anchorage-independently, although it does possess the capability to form colonies in semisolid media. This makes it an invaluable model for exploring cellular processes and transformations in a controlled environment. In contrast, its derivative lines such as those transformed by 3-Methylcholanthrene epoxide, MNNG, and SV40 demonstrate the ability to grow in soft agar and form tumors in immunodeficient mice, highlighting the impact of genetic and environmental alterations on cellular behavior. The BNL CL.2 cell line and its derivatives continue to provide a robust foundation for research in cellular transformation, stable cell transfection, and related fields of cellular and molecular biology.

Organism Mouse**Tissue** Liver**Synonyms** BNL-CL.2, BNL CL2, BNL.CL2, BN-CL2, BNCL-2, BNCL2**Characteristics****Breed/Subspecies** BALB/c**Age** Embryo**Morphology** Epithelial**Growth properties** Adherent**Regulatory Data****Citation** BNL CL.2 (Cytion catalog number 305177)**Biosafety level** 1**NCBI_TaxID** 10090

BNL CL.2 Cells | 305177**CellosaurusAccession** CVCL_4383**Biomolecular Data****Tumorigenic** No, the cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium.**Handling****Culture Medium** DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO₃, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)**Supplements** Supplement the medium with 10% FBS**Dissociation Reagent** Accutase**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.**Fluid renewal** 2 to 3 times per week**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

BNL CL.2 Cells | 305177

Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Incubation Atmosphere

37°C, 5% CO₂, humidified atmosphere.

Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately -78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about -150 to -196 °C. Storage at -80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

BNL CL.2 Cells | 305177

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.