Product sheet

NRK-4xlambdaN22-3xmEGFP-M9 Cells | 500672

General information

Description	The NRK-4xlambdaN22-3xmEGFP-M9 cell line is a clonal stable cell line derived from normal rat kidney (NRK) cells through the transfection of a circular plasmid. This plasmid contains genetic constructs encoding four tandem repeats of lambda N22 RNA-binding sites and three tandem repeats of mEGFP (monomeric enhanced green fluorescent protein) tags fused with the M9 nuclear localization signal. Post-transfection, the cells underwent drug resistance selection to ensure the stability of the genetic modifications. Approximately 50% of the cells in this clonal stable line express the fluorescent marker 4xλN22-3xmEGFP-M9, indicating successful incorporation of the plasmid. The expression of this marker allows for real-time visualization of intracellular processes, facilitated by the robust fluorescent signal of mEGFP. The M9 nuclear localization signal ensures that the expressed fusion proteins are transported to the nucleus, making this cell line particularly useful for studying nuclear-cytoplasmic transport, RNA dynamics, and gene expression regulation. This NRK-4xlambdaN22-3xmEGFP-M9 cell line is valuable for researchers focusing on RNA-binding protein interactions, RNA metabolism, and the mechanisms underlying nuclear import and export. The presence of the mEGFP marker enables advanced imaging techniques such as confocal microscopy and live-cell imaging, providing detailed insights into the spatial and temporal dynamics of cellular components. Despite the variegation, the cell line remains a powerful tool for dissecting complex molecular pathways and understanding cellular functions at a deeper level.
Organism	Rat
Tissue	Kidney
Synonyms	NRK 4xlN22-3xmEGFP-M9

Characteristics

Breed/Subspecies	OsborneMendel
Morphology	Fibroblast-like cells with fusiform shape
Growth properties	Monolayer, adherent

Regulatory Data

Citation	NRK-4xlambdaN22-3xmEGFP-M9 (Cytion catalog number 500672)
Biosafety level	1

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NCBI_TaxID	10116
CellosaurusAccession	CVCL_AV97
Depositor	The Ellenberg Lab (EMBL)

Biomolecular Data

Receptors expressed	Epidermal growth factor (EGF), multiplication stimulating activity (MSA)
Protein expression	4xλN22-3xmEGFP-M9: Location/Gene: 937..1009, 1066..1138, 1194..1261, 1323..1390 / lambda peptide, 1462..2176, 2179..2890, 2896..3612 / mEGFP, 3612..3815 / M9-His, 5090..5884 / KanR/NeoR, 7195..584 / Pcmv
Products	M9-His tag between BsrG1/HindIII, Neomycin, Phosphotransferase, CMV Promotor

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, 0.5 mg/mL G418
Dissociation Reagent	Accutase
Subculturing	Discard the old medium and wash the cells with PBS. Add a freshly prepared 0.025% trypsin/0.02% EDTA solution heated to 37 degrees Celsius and wait until the cells detach, which usually takes about 5 minutes. Neutralize the trypsin by adding fresh medium, then transfer the cell mixture to a tube and centrifuge. After centrifugation, remove the supernatant, resuspend the cell pellet in fresh culture medium, and transfer the suspension to new flasks. Incorporate G418 into the culture medium to achieve a final concentration of 0.5 mg/ml
Seeding density	2 to 4 x 10^4 cells/cm^2
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.

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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% CO_2, 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

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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.