

3D4/21 Cells | 305357**General information****Description**

The 3D4/21 cell line is a porcine alveolar macrophage model extensively used in studies involving immune responses and viral infections. These cells are particularly valuable for research on respiratory infections in pigs, such as those caused by swine influenza virus (SIV) and Senecavirus A (SVA). Due to their origin from alveolar macrophages, 3D4/21 cells serve as a critical in vitro system for examining host-pathogen interactions, focusing on the immune mechanisms that pigs deploy against respiratory pathogens.

3D4/21 cells are often utilized to explore the molecular pathways triggered during infections. For example, in the context of SIV infections, studies have shown that 3D4/21 cells mount an immune response through the expression of various cytokines and viral recognition receptors like RIG-I and TLR7. Furthermore, these cells have been instrumental in identifying how different RNA molecules, including miRNAs, lncRNAs, and circRNAs, contribute to the regulatory networks during viral infections, providing insights into the competitive endogenous RNA (ceRNA) regulatory mechanisms that help in antiviral defenses. This has been particularly useful in elucidating the immune responses triggered by different strains of swine influenza viruses such as H1N1 and H3N2.

In addition, 3D4/21 cells have been employed to study autophagy and its role in infection control. For example, during *Haemophilus parasuis* infection, these cells exhibit autophagic responses regulated via the AMPK signaling pathway. This autophagy mechanism not only contributes to controlling bacterial invasion but also helps in understanding the pathogenesis of diseases like Glässer's disease, a condition affecting pigs under stress. As a result, the 3D4/21 cell line continues to be a valuable tool for immunological and infectious disease research in the context of swine health.

Organism

Pig

Tissue

Lung

Synonyms

IPAM 3D4/21, IPAM-WT

Characteristics**Breed/Subspecies**

Landrace

Age

12 weeks

Gender

Unspecified

Morphology

Macrophage

Cell type

Alveolar macrophage

Growth properties

Adherent

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Citation	3D4/21 (Cytion catalog number 305357)
Biosafety level	2
NCBI_TaxID	9823
CellosaurusAccession	CVCL_0F14

Biomolecular Data

Viruses	Transformant: Simian virus 40 (SV40)
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Handling

Culture Medium	RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO ₃ (Cytion article number 820700a)
Supplements	Supplement the medium with 10% FBS
Dissociation Reagent	Accutase
Seeding density	1-3*10 ⁴ /cm ²
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% 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

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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.