# Certificate of Analysis

## Ensuring Integrity in Every Cell Line

| | | | |
|---|---|---|---|
| **Product** | 4T1 | **Passage Number** | 2 |
| **Species** | Mouse | **Cell Count per mL** | 2.0 million |
| **Cytion™ Number** | 300300 | **Volume per ampule** | 1.5 |
| **Lot Number** | 300300-291124 | **Freeze Date** | 29.11.2024 |
| **Product Format** | cryovial | **Freeze Medium** | Freeze medium containing FBS |
| **Storage Conditions** | Below -150°C | **Expiration Date** | Not applicable |

At Cytion, we ensure the quality and reliability of our cell lines through in-house and third-party testing, including:

1. **STR Profiling:** To authenticate cell line[1] identity
2. **Virus Testing:** For species-specific[2] viruses where possible
3. **Mycoplasma Testing:** To prevent contamination.
4. **Interspecies Contamination Testing:** To ensure purity.
5. **Bacteria and Fungi Testing:** To ensure cells are free from microbial contaminants.

1: Human, Mouse, Rat, Hamster and Canine Species.
2: Human, Mouse, Rat, Hamster, Bovine, and Porcine Species.

| Test Overview | Methodology | Acceptance Criteria | Outcome |
|---|---|---|---|
| **Post-Freeze Viability** | Viability assessment using Vi-CELL BLU by Beckman Coulter (analyzing 100 images per sample, 200 µl cell suspension per test) | ≥70% post-thaw cell viability | 81.8% |
| **Recovery** | Culture in the appropriate growth medium, microscopic evaluation | > 80% confluence within the expected recovery period | A T25 culture flask seeded at a density of 4.16 x $10^4$ viable cells per $cm^2$ typically reaches approximately 95% confluence within 3 days. |
| **Growth Properties** | Observation of cell culture behaviour to determine if cells are adherent, suspension, or semi-adherent | Cells exhibit expected growth properties consistent with the cell line's characteristics | Adherent |
| **Sterility Testing** | Visual inspection | Absence of microbial growth (bacterial, fungal, or yeast) | Pass |
| **Mycoplasma Testing** | PCR-based detection assay with specific primers targeting Mycoplasma DNA | No detectable Mycoplasma contamination confirmed by PCR | Pass |



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| | Marker | Alleles |
|---|---|---|
| Authentication | M_18-3 | 18 |
| | M_4-2 | 21.3 |
| | M_6-7 | 12 |
| | M_3-2 | 14, 15 |
| | M_19-2 | 13 |
| | M_7-1 | 25.2 |
| | M_1-1 | 16 |
| | M_Sex | X |
| | M_8-1 | 13 |
| | M_2-1 | 16, 17 |
| | M_15-3 | 22.3 |
| | M_6-4 | 18 |
| | M_11-2 | 18, 19, 20 |
| | M_1-2 | 17 |
| | M_17-2 | 15 |
| | M_12-1 | 16 |
| | M_5-5 | 14 |
| | M_X-1 | 25 |
| | M_13-1 | 16.2 |
| | Human D4/D8 | - |
| | Identity match | 94.6% |

| | Pathogen | Result |
|---|---|---|
| Species-Specific Pathogen Testing | Ectromelia | - |
| | EDIM | - |
| | LCMV | - |
| | LDEV | - |
| | MAV1 | - |
| | MAV2 | - |
| | mCMV | - |




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| | Pathogen | Result |
|---|---|---|
| **Species-Specific Pathogen Testing** | MHV | - |
| | MNV | - |
| | Mycoplasma pulmonis | - |
| | MVM | - |
| | MPV | - |
| | Polyoma | - |
| | PVM | - |
| | REO3 | - |
| | Sendai | - |
| | TMEV | - |




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**Third-Party Certified Quality Assurance**

Since 2023, IDEXX has conducted STR profiling, species-specific pathogen testing, and species contamination analysis to ensure the authenticity, purity, and safety of our cell lines.

IDEXX BioAnalytics

The information provided is believed to be accurate, but both the information and product are offered without warranty for any applications beyond those specified. This certificate confirms that the material has been tested and is pure to the best of our knowledge.

This product is intended solely for laboratory research use and is not for use in humans, animals, or diagnostics. Proper Biosafety Level practices are required.

Cytion products may not be resold, modified for resale, or used commercially without prior written consent from Cytion.

Manufactured in Germany under our ISO 9001:2015 certified Quality Management System, ensuring high standards of quality and reliability.

Dr. R. Steubing

ID: 72E76512-B2D5-470D-9223-9D0295A18D69
Reason: Approved <r.steubing@cytion.com>
April 09, 2025 02:05 PM CEST

Qualified personnel have validated the test results of this Certificate of Analysis and attested with their signature that the tests were conducted as reflected in the results presented here.



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