

THP1-Dual™ KO-MDA5 Cells

MDA5 knockout NF-κB-SEAP & IRF-Lucia luciferase reporter monocytes

Catalog code: thpd-komda5

<https://www.invivogen.com/thp1-dual-ko-rlr>

For research use only

Version 21C25-NJ

PRODUCT INFORMATION

Contents

• 3-7 x 10⁶ THP1-Dual™ KO-MDA5 cells in a cryovial or shipping flask. **IMPORTANT:** If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- 1 ml of **Blasticidin** (10 mg/ml). Store at 4°C or -20 °C.*
- 1 ml of **Zeocin**™ (100 mg/ml). Store at 4°C or -20 °C.*
- 1 ml of **Normocin**™ (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

• 1 pouch of **QUANTI-Luc**™ (Lucia luciferase detection reagent). Store pouch at -20°C. Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Protect from light.

• 1 ml of **QB reagent** and 1 ml of **QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue**™ Solution, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20°C. QUANTI-Blue™ Solution is stable for 2 weeks at 4°C and for 2 months at -20°C.

Note: Data sheets for all components are available on our website

Handling Frozen Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

Note: Avoid freezing cells upon receipt as it may result in irreversible damage to the cell line.

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures. **IMPORTANT:** For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

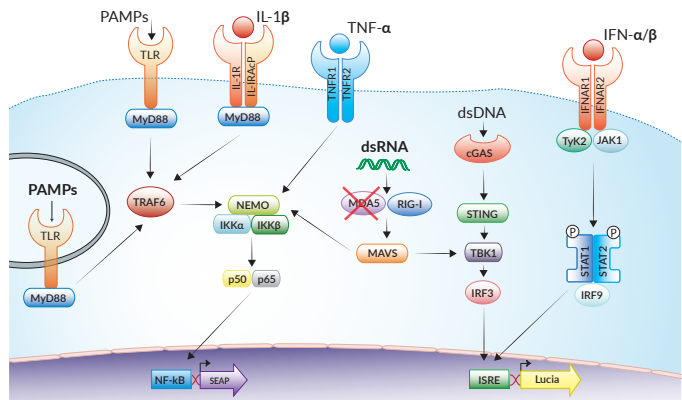
- Biallelic MDA5 gene knockout has been verified by PCR, DNA sequencing, and functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

APPLICATIONS

THP1-Dual™ KO-MDA5 are ideal for studying cytosolic RNA sensing pathways. These cells are also useful for investigating the crosstalk in RNA and DNA detection.

PRODUCT DESCRIPTION

THP1-Dual™ KO-MDA5 cells were generated from the THP1-Dual™ cell line, which is derived from the human THP-1 monocytes, through the stable knockout (KO) of the MDA5 gene. These cells feature two reporter genes allowing the simultaneous study of the IRF pathway, by monitoring the activity of an inducible secreted Lucia luciferase, and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). Lucia luciferase and SEAP activities are readily assessable in the supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively. As expected, the IRF and NF-κB responses are severely impaired in THP1-Dual™ KO-MDA5 cells upon incubation with Poly(I:C) HMW, a RLR inducer. These cells retain the ability to respond to other NF-κB activating ligands such as recombinant human TNF-α, as well as IRF-activating ligands such as type I interferons (IFNs). THP1-Dual™ KO-MDA5 cells are selectable with Blasticidin and Zeocin™.



BACKGROUND

MDA5 (melanoma-differentiation-associated gene 5, also known as Ifih1 or Helicard) is a cytoplasmic RNA helicase that plays an important role in antiviral response. It senses long double-stranded RNA (dsRNA), a replication intermediate for RNA viruses, leading to production of type I interferons (IFNs)¹. MDA-5 and the related RNA helicase RIG-I recognize a complementary set of cytosolic viral dsRNA. Of note, MDA5 has been described as a major sensor of SARS-CoV-2 infection, triggering the interferon response in lung cells². Transfected poly(I:C), a synthetic analog of viral dsRNA, is recognized by both MDA-5 and RIG-I.

1. Reikine S, et al., 2014. Pattern Recognition and Signaling Mechanisms of RIG-I and MDA5. Front Immunol. 5:342. 2. Yin X, et al., 2021. MDA5 governs the innate immune response to SARS-CoV-2 in lung epithelial cells. Cell Reports. 34:108628..

TECHNICAL SUPPORT

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Any questions about our cell lines?
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www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml **Normocin™**

Initial culture of all THP-1 derived cells must be performed in growth medium containing 20% heat-inactivated FBS.

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate TLRs resulting in differentiation of the monocytes and activation of the reporter gene.

• **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin

• **Freezing Medium:** 95% FBS and 5% DMSO

Required Selection Antibiotics

• **Blasticidin** and **Zeocin™**

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS). **Do not add selective antibiotics until the cells have been passaged twice.**

4. Centrifuge at 150 x g (RCF) for 10 minutes.

5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).

6. Transfer the cells to a T-25 culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).

7. Place the culture at 37°C in 5% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freezing medium freshly prepared with cold FBS.

Note: A T-75 culture flask yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of the cell suspension into cryogenic vials.

3. Place vials in a freezing container and store at -80°C overnight.

4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. THP1-Dual™ KO-MDA5 cells grow in suspension.

2. After cells have recovered and are growing well (following at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 10 µg/ml **Blasticidin** and 100 µg/ml **Zeocin™** to the growth medium every other passage.

3. Pass the cells every 3 days by inoculating 5 x 10⁵ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees with the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

REPORTER ASSAY

To ensure the best results, use THP1-Dual™ KO-MDA5 cells with less than 20 passages.

Cell preparation

1. Centrifuge at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
2. Remove supernatant and resuspend THP1-Dual™ KO-MDA5 cells at 5 x 10⁵ cells/ml in freshly prepared, pre-warmed test medium.

Detection of NF-κB induction

1. Add 20 µl of test compound per well of a flat-bottom 96-well plate. Include a positive control (such as **recombinant human TFN-α** at 3 ng/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).

2. Add 180 µl of cell suspension (~100,000 cells) per well.

3. Incubate the plate for 18-24h at 37°C, 5% CO₂.

4. Prepare **QUANTI-Blue™ Solution** following the instructions on the enclosed data sheet.

5. Dispense 180 µl of **QUANTI-Blue™ Solution** per well of a new flat-bottom 96-well plate.

6. Add 20 µl of stimulated THP1-Dual™ KO-MDA5 cell culture supernatant per well.

7. Incubate the plate at 37°C for 1-3 h.

8. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Detection of IRF induction

Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Add 20 µl of test compound per well of a flat-bottom 96-well plate. Include a positive control (such as recombinant human IFN-β at 1000 IU/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).

2. Add 180 µl of cell suspension (~100,000 cells) per well.

3. Incubate the plate for 18-24h at 37°C, 5% CO₂.

4. Prepare **QUANTI-Luc™** following the instructions on the enclosed data sheet.

5. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.

6. Add 10 µl of stimulated THP1-Dual™ KO-MDA5 cell culture supernatant per well into a 96-well white (opaque) or black plate, or a luminometer tube.

7. Prime the injector with the **QUANTI-Luc™** assay solution and proceed with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
Blasticidin	Selection antibiotic	ant-bl-1
Zeocin™	Selection antibiotic	ant-zn-1
Poly(I:C) HMW	RLR ligand	tlrl-pic
3p-hpRNA/LV	RIG-I ligand	tlrl-hprnalv

TECHNICAL SUPPORT

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QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2

<https://www.invivogen.com/quantiluc>

For research use only

Version 19A04-MM

PRODUCT INFORMATION

Contents

QUANTI-Luc™ is provided as packs of individually sealed pouches.

- rep-qlc1: 2 pouches of QUANTI-Luc™
- rep-qlc2: 5 pouches of QUANTI-Luc™

Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability

- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

DESCRIPTION

QUANTI-Luc™ is an assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS

Preparation of QUANTI-Luc™

1. Pour the pouch contents into a 50 ml screw cap tube.
2. Add 25 ml of sterile water.
3. Swirl product gently until powder is completely dissolved.
4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

Detection of luciferase activity from cell culture medium

To obtain **end-point readings** using a luminometer **with an injector**.

1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with the QUANTI-Luc™ assay solution and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.

1. Set the luminometer with a 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ assay solution to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™ Gold (For standard and HTS assays)	rep-qlcg1
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
Recombinant Lucia luciferase protein	rec-lucia
Reporter Cells	
THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-κB Cells	thp1-nfkb

For a complete list of InvivoGen's Lucia luciferase Reporter Cell Lines visit <https://www.invivogen.com/lucia-reporter-cells>.

TECHNICAL SUPPORT

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QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2, rep-qbs3

<https://www.invivogen.com/quant-blue>

For research use only

Version 20C16-MM

PRODUCT INFORMATION

Contents: QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).

- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).

- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

Required Material (not provided)

- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and stability

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.

- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles.**

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent. If this occurs, vortex the product until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect QUANTI-Blue™ from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (including pH, solubility).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP. Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a glycosylphosphatidylinositol (GPI)-anchored protein. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters.

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

TECHNICAL SUPPORT

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METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

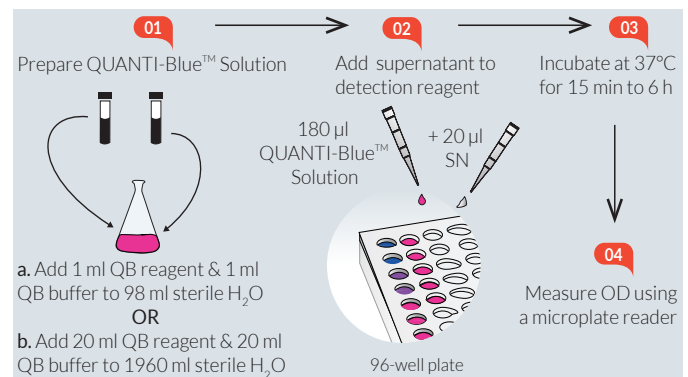


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.

1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
 - a. 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
 - b. 20 ml of QB reagent and 20 ml of QB buffer to 1960 ml of sterile water.
2. Mix by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of the sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

B. High Throughput Screening (HTS) procedure

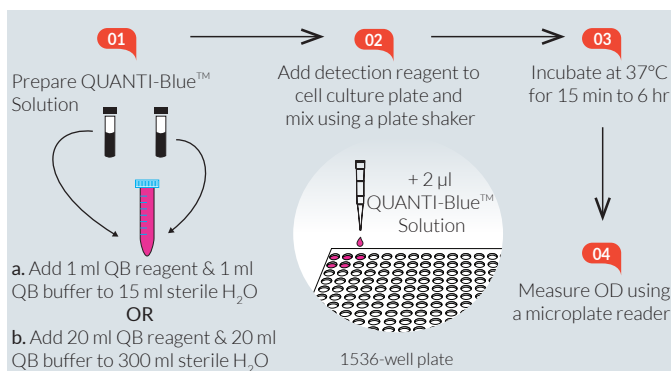


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling.

Ensure QB reagent and QB buffer are completely thawed before use. *Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.*

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed **5 µl** per well. Incubate cells with test compounds for the desired period of time.
2. Prepare QUANTI-Blue™ Solution by adding:
 - a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
 - b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
5. Dispense **2 µl** of QUANTI-Blue™ Solution to the wells containing $\leq 5 \mu\text{l}$ of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37°C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNifTy2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit <https://www.invivogen.com/reporter-cells>

TECHNICAL SUPPORT

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