Applied Biological Materials Inc.
Telephone: 1-866-757-2414
Email: info@abmgood.com
Website: www.abmgood.com
Version 1: May 25th, 2026

Human Kidney Organoid Differentiation Medium Kit

Cat. No. TM209
Store at -20°C.

Product Description
The Human Kidney Organoid Differentiation Medium Kit is a 5-stage differentiation system designed to support the generation and maturation of kidney organoids from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs). The kit enables reproducible induction, patterning, and maturation of nephron-like structures using stage-specific medium formulations.

Included Media Components
1. **Medium 1 – Induction**
   Initiates early lineage commitment and drives cells toward renal fate.
2. **Medium 2 – Specification**
   Promotes intermediate mesoderm specification and early renal progenitor development.
3. **Medium 3 – Patterning / Stabilization**
   Supports spatial organization and nephron lineage patterning within 3D culture.
4. **Medium 4 – Nephron Induction**
   Facilitates nephron segmentation and structural differentiation of renal subtypes.
5. **Medium 5 – Maintenance**
   Enhances functional maturation and supports long-term stability of kidney organoids.

Key Features
- Stage-specific kidney organoid differentiation system
- Supports transition from monolayer differentiation to 3D organoid culture
- Supports nephron-like structure formation
- Compatible with both hESC- and hiPSC-based workflows
- Ready-to-use medium system

The Human Kidney Organoid Differentiation Medium Kit enables researchers to efficiently generate and mature complex renal organoids for disease modeling, drug screening, and mechanistic studies. The kit is comprised of the following components:

Product Component	Part No.	Quantity	Storage Condition
Medium 1 – Induction	TM209-1	50 ml	-20°C
Medium 2 – Specification	TM209-2	25 ml	-20°C
Medium 3 – Patterning / Stabilization	TM209-3	40 ml	-20°C
Medium 4 – Nephron Induction	TM209-4	20 ml	-20°C
Medium 5 – Maintenance *(Human Kidney Organoid Maintenance Medium)*	TM210	100 ml	-20°C

Additional required materials (not included in kit)

- Human ESCs or iPSCs
- Stem cell growth medium (i.e. mTeSR, E8 etc)
- StemRelease™ (TM097)
- ROCK Inhibitor Y-27632 (TM131)
- 3DCelMatrix™ (TM076)
- SpheroWell™ 6 Well Plate (G7541)
- 1X DPBS, No Ca, No Mg (CH110)
- Wide-bore pipette tips

Protocol for Kidney Organoid Differentiation

The following protocol is optimized for generating human kidney organoids in a 6-well plate format. Reagent volumes may be adjusted proportionally based on the culture vessel size used.

Unless otherwise specified, all cultures were maintained at 37°C with 5% CO_2.

Day -3 to Day 0 — Recovery and Preparation of Human ESCs/iPSCs for Kidney Organoid Differentiation

Before initiating differentiation, passage and culture the stem cells for at least three passages to ensure the cells are fully recovered, healthy, free of mycoplasma contamination, and exhibit stable growth and doubling rates.

Initiate culture of human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

1. Prepare a 1% 3DCelMatrix™ coated 6-well plate.
2. Add 2.0 ml of appropriate stem cell growth medium (e.g. mTeSR™ Plus or Essential 8 medium).
3. Seed human ESCs or iPSCs onto the coated 6-well plate at approximately 15% confluency.
4. Replace medium daily and maintain cells until Day 0, ensuring the cell confluency reaches approximately 40–50%.
5. Once cells reach 40–50% confluency with healthy morphology, proceed with differentiation.

Day 0–3. Mesoderm Induction

1. Aspirate culture medium and wash cells once with 1X DPBS without Ca^{2+} and Mg^{2+} to remove residual growth factors that may interfere with differentiation.
2. Add 2.0 ml of Medium 1 per well.
3. Maintain cells in Medium 1 from Day 0 to Day 3, replacing medium every other day (every 48 hours).
4. Transient cell loss during the first 4 days is expected and is commonly associated with successful mesoderm lineage induction.
5. By Day 3, cells should form a dense epithelial-like monolayer. Proceed to the next step.

Day 4–6. Intermediate Mesoderm Patterning

1. Aspirate Medium 1 and wash cells once with 1X DPBS without Ca^{2+} and Mg^{2+} to remove residual medium.
2. Add 2.0 ml of Medium 2 per well.
3. Maintain cells in Medium 2 until Day 6 with medium changes every 48 hours.

Day 7–8. Nephron Patterning

1. On Day 7, aspirate Medium 2 and add 2.0 ml of Medium 3 per well.
2. Maintain cells in Medium 3 until Day 8 with medium changes every 48 hours.

Day 9–10. Nephron Induction

1. On Day 9, aspirate Medium 3 and add 2.0 ml of Medium 4 per well.
2. Maintain cells in Medium 4 until Day 10 with medium changes every 48 hours.

Day 11–13. Stabilization

1. On Day 11, aspirate Medium 4 and add 2.0 ml of Medium 3 per well.
2. Maintain cells in Medium 3 until Day 13 with medium changes every 48 hours.

Day 14–21+. Maintenance and Maturation

1. By Day 14, distinct nephron segmentation and organized nephron-like structures should be clearly visible.
2. Aspirate Medium 3 and add 2.0 ml of Medium 5 per well.
3. Organoids can be collected and transitioned to 3D suspension culture (see Transition from 2D to 3D Culture Protocol). Maintain organoids in Medium 5.

4. Replace maintenance medium every other day (every 48 hours) and continue culture until approximately Day 28, when mature kidney organoids are suitable for downstream experimental applications and research studies.

Transition from 2D to 3D Culture Protocol

Below is a general procedure for transitioning kidney organoids from 2D differentiation culture into 3D suspension culture.

1. Once nephron-like structures are visible, gently dislodge organoids using a wide-bore pipette tip.
2. Transfer organoids into a SpheroWell™ 6-Well Plate containing Medium 5 supplemented with 10 µM ROCK Inhibitor Y-27632.
3. Maintain organoids under gentle orbital shaking culture conditions.
4. Replace medium after 24 hours to remove ROCK Inhibitor.
5. Continue maintenance culture in Medium 5 with medium changes every 48 hours until mature kidney organoids are formed.

Long-term Culture

Kidney organoids may be maintained beyond Day 28 with continued Medium 5 changes every 48 hours. Functional characteristics and morphology should be monitored regularly for extended culture experiments.

General Notes

- Do not allow medium pH to become acidic during culture.
- Always use wide-bore pipette tips when handling organoids.
- Avoid excessive mechanical disruption during transfer.
- Healthy kidney organoids should exhibit compact morphology with visible nephron-like structures.
- Mature kidney organoids are typically observed between Day 21–28.

For research use only. Not intended for therapeutic or diagnostic use.