1. tPGC; Chicken

Product name: **Tissue Primordial Germ Cells; Chicken**

Catalog No. CELL-00001

Tissue primordial germ cells are germ cell progenitors isolated from the whole embryo during pre-gonadal stage of primordial germ cells *in vivo*. The cells are characterized by positive expression of germ cell-specific protein markers and *in vivo* gonadal migration assay.

Product category	Animal cells
Organism	*Gallus gallus domesticus*, chicken
Cell type	Primordial germ cells
Product type	Stem cells
Morphology	spherical colony
Tissue	Embryo; Whole
Product format	Frozen
Storage conditions	Liquid nitrogen

General	
Applications	Germ cell differentiation, germline genome engineering, assisted reproductive technologies
Characteristics	
Cells per vial	50,000 –100,000
Volume	1.0 mL
Growth properties	Loosely adherent on feeder cells
Age	Embryonic, 2 d
Breed	Local breeds
Sex	Lot-specific, female and male available
Remarks	The cells are cryopreserved in the first or second passage to ensure high plating efficiency.

Handling	
Unpacking	Upon arrival, ensure that containers and frozen vials are intact. Retrieve the frozen cells from the packaging and immediately place the cells in liquid nitrogen storage until ready for use.
Complete medium	MEM α (Gibco Catalog No. 11900-024) containing 1.2 g/L D-glucose, 2.52 g/L EPPS and 1.1 g/L sodium carbonate supplemented with 2-mercaptoethanol (1000X) (Gibco Catalog No. 21985023), Antibiotic-Antimycotic (100X) (Gibco Catalog No. 15240062), 5% chicken serum (Sigma Catalog No. C5405) and 5% fetal bovine serum (Cytiva Catalog No. SV30160).
Temperature	37°C
Atmosphere	Air
Feeder cells	Tissue PGC are propagated on feeder cells. Japanese quail feeder cells (Visit the product page of JQ Feeder cells) are tested for propagation of tissue PGC from various avian species. Feeder cells are grown in the same medium as that of tissue PGC. Consult the product detail of the JQ feeder cells for subculture and mitotic inhibition protocols (Visit the product page of JQ Feeder cells).
Handling procedure	Tissue PGC are propagated on feeder cells, therefore mitotic-inhibited feeder cells are required before inoculating with tissue PGC. Most of the cells are frozen in clumps since this is the general pattern of tissue PGC growth in KAv-1 medium (refer to references). The cells are likely to resume to grow in clumps, although the growth in single cells is frequently observed in some lots. 1. Plate mitotic-inhibited JQ cells a feeder layer onto a T_{25} flask at least one day before thawing the tissue PGC vial. Consult the product detail of the JQ feeder cells for subculture, mitotic inhibition, and plating protocols. Renew the culture medium 100% just prior to thawing the vial. 2. Aliquot 9 mL of fresh culture medium into a 15 mL centrifugal tube.

3. Retrieve the frozen vial from the liquid nitrogen storage and thaw the vial by gentle agitation in a water bath at 37°C. Although the internal-thread vial is used, keep the cap out of the water to prevent contamination into the cells. Thawing is achieved in approximately one minute, do not completely thaw the cells in the water bath.
4. Transfer the thawed content by slow dropping it into a centrifugal tube containing 9 mL of culture medium prepared in Step 2.
5. Centrifuge the cell suspension at 400 x g at 4°C for 5 minutes.
6. Aspirate the supernatant and resuspend the cell pellet in 1 mL of complete medium. Gently break the clumps into smaller colonies by using a P1000 pipette.
7. Transfer the cell suspension into a T_{25} flask containing a feeder layer and fresh culture medium. Distribute the cells evenly in the flask.
8. Incubate the culture at 37°C.

Maintenance

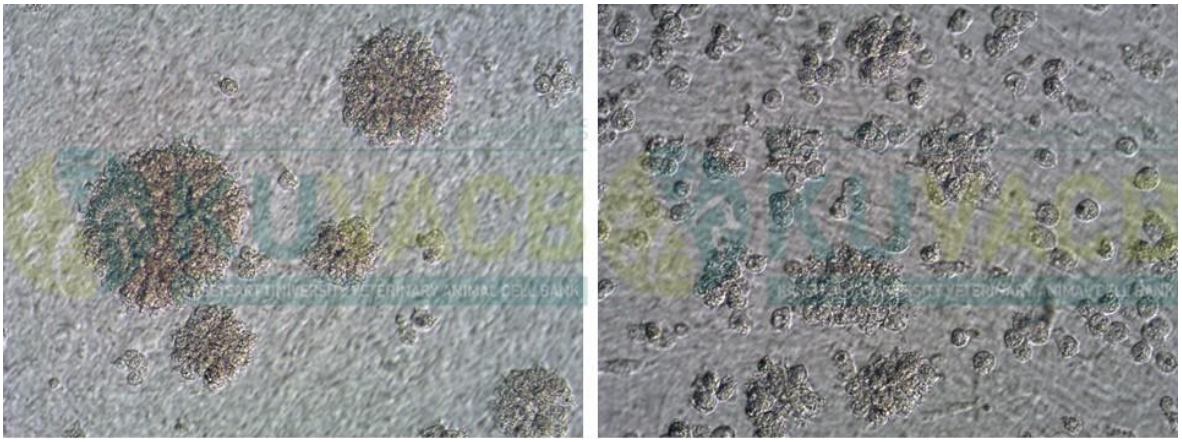
1. Observe the appearance of spherical colonies comprising of large round cells with refractive granules under an inverted microscope. The cells exhibit several cytoplasmic spikes, a distinctive feature of tissue PGC (Refer to the Image section).
2. Renew half of the spent medium every two days. Carefully remove the spent medium without agitating the monolayer because the cells are only loosely attached to feeder cells, especially when they are freshly inoculated. Suspension of large colonies in the medium is frequently observed. Culturing in T_{25} flask requires 6 mL of complete medium.
3. Observe the confluence that necessitates the subculture. Large colonies can often form in some well-proliferated cultures. *Note: Overgrowth of colonies will result in the appearance of membrane blebs and cell death.*

Subculture procedure	After recovery from cryopreservation, it may take one to two weeks for the cells to proliferate and are ready for the subculture. The subculture ratio depends on the confluency of the colonies which been achieved by 1:2 or 1:3 ratio. Determine the ratio and the number of feeder-covered flasks required and plating the feeder cells into a new flask at least one day before the subculture. Cell dissociation by gentle pipetting does not result in low viability or cell death. 1. Collect half of the spent medium carefully by using a transfer pipette and transfer the medium into a 15 mL centrifugal tube. This 'conditioned medium' is to be used in conjunction with the fresh medium when adding the cell suspension into a new flask. 2. Detach the cells from the feeder layer by tapping the flask or gentle pipetting. Wash off gently with 1–2 mL of culture medium if some colonies remain. Avoid dislodging the feeder layer along with the cells. 3. Collect the cell suspension into a new 15 mL centrifugal tube. 4. Centrifuge the cell suspension at 400 x g at 4°C for 5 minutes. 5. Aspirate the supernatant and resuspend the cell pellet in 1 mL of complete medium. Gently break the clumps into smaller colonies by using a P1000 pipette. 6. Transfer the cell suspension into a T_{25} flask covered with feeder cells. Distribute the cells evenly in the flask. 7. Add the condition medium collected in Step 1, then add the equal amount of fresh complete medium into the flask. 8. Incubate the culture at 37°C.
Cryopreservation	Cryopreservation medium: 90% FBS and 10% DMSO (freshly prepared). 1. Follow the Subculture procedure above to dissociate the cells.

2. Centrifuge the cell suspension at 400 x g at 4°C for 5 minutes.
3. Remove the supernatant and slowly resuspend the cell pellet with cryopreservation medium (1mL per vial). One to two vials can be achieved from one T_{25} culture.
4. Evenly distribute 1 mL of cell suspension into each cryovial.
5. Transfer the cryovials into a freezing container and store at -80°C overnight, then transfer the vials to liquid nitrogen storage.

Quality controls	
Viability	≥ 50% post-thawed from cryopreservation
Specific staining	Positive expression for CVH and DAZL
Functional assays	Positive for *in vivo* gonadal migration assay

Image



References

Chaipipat, S., Prukudom, S., Sritabtim, K., Kuwana, T., Piyasanti, Y., Sinsiri, R., Piantham, C., Sangkalerd, S., Boonsanong, S., Pitiwong, K., Pidthong, A., Wanghongsa, S., & Siripattarapravat, K. (2021). Primordial germ cells isolated from individual embryos of

red junglefowl and indigenous pheasants of Thailand. *Theriogenology*, *165*, 59-68. https://doi.org/10.1016/j.theriogenology.2021.02.010

Chaipipat, S., Sritabtim, K., Piyasanti, Y., Prukudom, S., Jurutha, J., Phetpila, V., Sinsiri, R., Kammongkun, J., Molee, A., Thiangtum, K., & Siripattarapravat, K. (2023). Initiative on Avian Primordial Germ Cell Cryobanking in Thailand. *Biopreserv Biobank*, *21*(5), 458-466. https://doi.org/10.1089/bio.2022.0043

Sritabtim, K., Prukudom, S., Piyasanti, Y., Chaipipat, S., Kuwana, T., Jurutha, J., Sinsiri, R., Tirawattanawanich, C., & Siripattarapravat, K. (2024). First study on repeatable culture of primordial germ cells from various embryonic regions with giant feeder cells in Japanese quail (Coturnix japonica). *Theriogenology*, *213*, 43-51. https://doi.org/10.1016/j.theriogenology.2023.09.020