



## **Chicken Primordial Germ Cell Collection from 5.5 Day Old Embryo Gonads and Subsequent Cryopreservation Protocol**

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Gonads are dissected from 5.5 day old chicken embryos and placed in 1.5ml siliconized tubes containing 500µl of 10% P-FBS DMEM. After pooling gonads from approximately 10 embryos the samples are centrifuged at 600 x g for 5 minutes. The gonad suspension is then diluted with 200µl of a 0.25% trypsin/EDTA (Gibco, Grand Island, NY) solution and incubated for 20 minutes at 37°C.

The gonad suspension is teased until only the homogenate remains, which is then subsequently removed. The trypsin is then deactivated by the addition of 200µl of 20% P-FBS DMEM. The cell suspension is then passed through a 30 micron filter that has been pre-wet with 50µl of 20% P-FBS DMEM and collected in a 1.5ml siliconized tube. The trypsin tube is rinsed twice with 200µl of 20% P-FBS DMEM and then passed through the filter as well. The tube is then washed twice with 200µl 0% FBS DMEM and the solutions are passed through the filter. The cell suspension is then centrifuged for 5 minutes at 600 x g. The supernatant is then removed and 500µl of 20%ES-FBS 199 is added to the cell suspension. The final cell concentration from 10 embryos should be approximately  $2 \times 10^6$  cells/ml.

The cell suspension is then cooled to 5°C over one hour and the remaining steps are performed at 5°C. The cell suspension is centrifuged for 5 minutes at 600 x g and the supernatant is removed. The cell suspension is diluted with 150µl of 5°C cryopreservation media, loaded into 0.5ml straws and heat sealed. The straws are frozen at a rate of -1°C/minute to -85°C using a programmable freezer (Mini Digitcool UJ40, Cryo Bio System, Paris, France) and then plunged into liquid nitrogen for storage.

Straws are thawed in a 37°C water bath for 30 seconds.

### **Recipes:**

#### **0% FBS DMEM**

1% Penicillin/Streptomycin  
99% Dulbecco's Modified Eagle's Medium

#### **10% P-FBS DMEM**

10% Premium Fetal Bovine Serum  
1% Penicillin/Streptomycin  
89% Dulbecco's Modified Eagle's Medium

#### **20% P-FBS DMEM**

20% Premium Fetal Bovine Serum  
1% Penicillin/Streptomycin  
79% Dulbecco's Modified Eagle's Medium

**20%ES-FBS 199**

20% Embryonic Stem Cell-Qualified Fetal Bovine Serum  
1% Penicillin/Streptomycin  
79% Media 199

**Cryopreservation media recipe:**

20% Embryonic Stem Cell-Qualified Fetal Bovine Serum  
1% Penicillin/Streptomycin  
10% Ethylene Glycol  
69% media 199

**References:**

Moore, D.T., P.H. Purdy, and H.D. Blackburn. 2006. A Method for Preserving Chicken Primordial Germ Cells. *Poult. Sci.* 85(10):1784-1790.

Mozdziak, P.E., J. Angerman-Stewart, B. Rushton, S.L. Pardue, and J.N. Petite. 2005. Isolation of chicken primordial germ cells using fluorescence-activated cell sorting. *Poult. Sci.* 84:594-600.