Diploid Growth Serum-Reduced Medium

Catalog Numbers A3968901, A3968902

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ Diploid Growth Serum-Reduced Medium (SRM) is designed to support vaccine manufacture with human diploid cells including MRC-5, WI-38, etc. The medium is available as a kit containing a basal medium (in dry powder format) as well as a 100X frozen liquid supplement. The Diploid Growth Serum-Reduced Medium (SRM) Kit is designed to support growth of human diploid cells with only 1% to 2% serum. It is also suitable for the growth of chicken embryo fibroblasts without serum supplementation. The Diploid Growth Supplement contains an animal-origin component from a BSE/TSE free source and is tested for viruses (9 CFR). When paired with Diploid Production Serum-Free Medium (SFM), the kit provides comparable growth and virus titers to conventional medium supplemented with 5–10% serum.

Contents and storage

Contents	Cat. No. A3968901	Cat. No. A3968902	Storage	Shelf life[1]
Diploid Growth Serum-Reduced Medium Kit:				
Diploid Basal Medium	10 L	100 L	2°C to 8°C. Protect from light.	12 months
Diploid Growth Supplement (100X)	100 mL	1000 mL	–5°C to –20°C. Protect from light.	

[1] Shelf-Life duration is determined from Date of Manufacture.

Culture conditions

Media: Diploid Growth Serum-Reduced Medium

Cell line(s): MRC-5, WI-38, 2BS, and CEF

Culture type: Adherent

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2 in air. Ensure proper gas exchange and minimize exposure of media and cultures to light.

Procedural guidelines

- Diploid Basal Medium contains 6 mM L-Glutamine. No additional supplementation is necessary.
- We recommend the use of Diploid Growth Serum-Reduced Medium with 1%–2% serum with human diploid cells.
- Diploid Growth Serum-Reduced Medium uses a sodium bicarbonate buffer system (2.2 g/L) and therefore requires 5–10% CO_2 environment to maintain physiological pH.
- We recommend nano-filtration of the growth supplement after thawing to ensure viral risk mitigation. The Planova™ 20N Virus Removal Filter (Asahi Kasei) has been shown to not have a detrimental impact on the performance of the supplement.

Reconstitute Diploid Basal Medium

1. Add 14.3 g/L of Diploid Basal Medium to 80% of the target reconstituted volume of deionized distilled water at 15°C to 30°C.
2. Mix for 30 minutes or until completely dissolved.
3. Add 2.2 g sodium bicarbonate ($NaHCO_3$, reagent grade) per liter of medium.

   Mix until dissolved.
4. Adjust pH of medium with 1 N NaOH or 1 N HCl to pH 7.10.

   Add dropwise with stirring and constant pH monitoring.
5. Use a calibrated vessel to dilute to final target volume with deionized distilled water.

   Mix for an additional 10 minutes.

6. Filter sterilize by 0.2 µm pore size membrane filtration immediately.

   Positive pressure filtration is recommended.

   Note: Store reconstituted Diploid Basal Medium at 2°C to 8°C protected from light.

Prepare complete Diploid Growth Serum-Reduced Medium

1. Thaw the frozen Diploid Growth Supplement (100X) at room temperature or overnight at 2°C to 8°C.

   IMPORTANT! Do not thaw the frozen supplement at 37°C. Avoid multiple freeze-thaw cycles of the Diploid Growth Supplement.

2. Mix the thawed supplement by gently inverting 3–5 times.

3. Aseptically transfer 10 mL of Diploid Growth Supplement (100X) to 1 L of Diploid Basal Medium.

   Additionally, 1% to 2% serum is also recommended for diploid cell growth applications.

4. Gently invert the bottle several times to obtain 1 L of homogeneous complete medium.

5. Store complete Diploid Growth Serum-Reduced Medium at 2°C to 8°C for up to 4 weeks.

6. Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch. Alternatively, an aliquot for use that day may be pre-warmed at 37°C until no longer cool to the touch. Avoid extended dwell times at 37°C.

   For chicken embryo fibroblasts, no serum supplementation is necessary.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath.

2. Transfer the entire contents of the cryovial into a T-75 flask containing 20 mL of prewarmed complete Diploid Growth Serum-Reduced Medium without antibiotics.

3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air. Use vented caps to allow for gas exchange.

4. Subculture when cells reach 80–100% confluency (1–2 days post thaw).

   Subculture cells a minimum of 3 passages before use in other applications.

Sub-culture in Diploid Growth Serum-Reduced Medium

Note: Volumes are recommendations for culture in T-75 flask.

1. Aspirate medium from cell monolayer and rinse flask with 10 mL prewarmed DPBS, no calcium, no magnesium. Aspirate DPBS.

2. Add 3 mL prewarmed TrypLE™ Express Enzyme or 0.05% Trypsin-EDTA to flask.

3. Incubate until cells have detached (~2–3 minutes at room temperature). Gently tap flask to dislodge cells.

4. Stop the dissociation reaction by adding 6 mL of Defined Trypsin Inhibitor to the flask. Triturate/pipette cell suspension to thoroughly rinse flask.

5. Transfer cell suspension to a sterile 15-mL centrifuge tube and centrifuge at 200 × *g* for 5 minutes.

6. Aspirate supernatant and resuspend the cell pellet in 5 mL prewarmed complete Diploid Growth Serum-Reduced Medium.

   If cell clumping is observed disperse cells by pipetting up and down until clumps are dispersed into a single cell suspension.

7. Determine total viable cell density.

8. Seed flasks at 1.5×10^4 viable cells/cm^2.

9. Incubate at 37°C in a humidified atmosphere of 5% CO_2 until cells are 70–90% confluent, usually 3–4 days post-seeding.

Adapt cultures to Diploid Growth Serum-Reduced Medium

Little or no adaptation is needed for human diploid cell lines, such as MRC-5 or WI-38.

Direct adaptation

It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation. Successful adaptation will depend upon the particular cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

1. Subculture cells grown in conventional medium with 5–10% serum into prewarmed complete Diploid Growth Serum-Reduced Medium.

2. Continue to monitor and passage cells for 2–3 passages until consistent growth is achieved.

   Note: If human diploid cells are seeded at high densities, they may exhibit contact inhibition. Evaluate growth performance at multiple seeding densities for optimal performance.

Cryopreservation

Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Save the conditioned medium to prepare cryopreservation medium.

1. Prepare desired quantity of cells in T-flask cultures, harvesting when cells reach approximately 80% confluency.

2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 1–5 × 10^6 cells/ mL.

3. Prepare the required volume of cryopreservation medium of 90% complete Diploid Growth Serum-Reduced Medium (50:50 ratio of fresh to conditioned media) + 10% DMSO on day of intended use, and store at 4°C until use.

   Note: Conditioned medium should be from day 2–4 cultures collected prior to subculture and trypsinization procedure.

4. Harvest cells (see "Sub-culture in Diploid Growth Serum-Reduced Medium" steps 1–4) and centrifuge at 100 × *g* for 5 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.

5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).

6. Cryopreserve in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).

7. Transfer frozen cells to liquid nitrogen (vapor phase); storage at –196°C to –125°C is recommended.

   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (See "Recovery").

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
DPBS, no calcium, no magnesium	14190
TrypLE™ Select Enzyme (1X), no phenol red	12563011
Trypsin-EDTA (0.05%)	25300
Defined Trypsin Inhibitor	R007100
Water For Injection (WFI) for Cell Culture	A12873
Diploid Production Serum-Free Medium Kit	A3969001 A3968902
Trypan Blue Stain	15250
Countess™ II Automated Cell Counter	AMQAX1000

Limited product warranty

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