


Diploid Production Serum-Free Medium (SFM)

Catalog Numbers A3969001, A3969002

Pub. No. MAN0019014 Rev. B.0

 **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](https://www.thermofisher.com/support).

Product description

Gibco™ Diploid Production Serum-Free Medium (SFM) 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 Production Serum-Free Medium (SFM) Kit is designed to support virus production with human diploid cells without serum supplementation. The Diploid Production Supplement is animal-origin component free. When paired with Diploid Growth Serum-Reduced Medium, the kit provides comparable growth and virus titers to conventional medium supplemented with 5–10% serum.

Contents and storage

Contents	Cat. No. A3969001	Cat. No. A3969002	Storage	Shelf life ^[1]
Diploid Production Serum-Free Medium Kit:				
Diploid Basal Medium	10 L	100 L	2°C to 8°C. Protect from light.	12 months
Diploid Production 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 Production Serum-Free 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% CO₂ 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 Production Serum-Free Medium without serum supplementation for virus production with human diploid cells.
- Diploid Production Serum-Free Medium uses a sodium bicarbonate buffer system (2.2 g/L) and therefore requires 5–10% CO₂ environment to maintain physiological pH.

Reconstitute Diploid Basal Medium

- 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.
 - Mix for 30 minutes or until completely dissolved.
 - Add 2.2 g sodium bicarbonate (NaHCO₃, reagent grade) per liter of medium.
Mix until dissolved.
 - Adjust pH of medium with 1 N NaOH or 1 N HCl to pH 7.10.
Add dropwise with stirring and constant pH monitoring.
 - Use a calibrated vessel to dilute to final target volume with deionized distilled water.
Mix for an additional 10 minutes.
 - 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 Production Serum-Free Medium

1. Thaw the frozen Diploid Production 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 Production Supplement.

2. Mix the thawed supplement by gently inverting 3–5 times.
3. Aseptically transfer 10 mL of Diploid Production Supplement (100X) to 1 L of Diploid Basal Medium.
4. Gently invert the bottle several times to obtain 1 L of homogeneous complete medium.
5. Store complete Diploid Production Serum-Free 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.

Prepare virus

1. Culture cells according to *Diploid Growth Serum Reduced Medium User Guide* (Pub. No. MAN0019015).
2. Once desired cell density has been reached, replace Diploid Growth Serum-Reduced Medium with Diploid Production Serum-Free Medium.

Note: A wash step before infection is not necessary.

Note: Multiplicity of infection, time of infection, and time of harvest may be different than with conventional media. Evaluate optimal conditions with Diploid Production Serum-Free Medium.

3. Incubate culture at desired temperature in a humidified atmosphere of 5% CO₂ in air. Use vented caps to allow for gas exchange.
4. Harvest virus when cytopathic effect is visible, or when harvest time point has been determined through other methods.

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 Growth Serum-Reduced Medium Kit	A3968901 A3968902

Limited product warranty

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