

## Advanced DMEM/F12

**Cat. No.: PM153312**

**Size: 500mL**

### General Information

Product Form	Liquid
Concentration	1 ×
pH	7.0-7.4
D-Glucose	3151 mg/L
L-Glutamine	365 mg/L
Sodium pyruvate	110 mg/L
Phenol red	8.1 mg/L
Non-Essential Amino Acids	Positive
HEPES	Negative
Osmotic pressure	300-340 mOsm/kg
Solvent	Purified water
Storage	2-8°C, Shading Light
Shipping	Ice bag
Expiration date	12 months

### Description

Advanced DMEM/F12 is a widely used basal medium that allows the culture of mammalian cells with reduced Fetal Bovine Serum (FBS) supplementation. Compared to classic DMEM/F12, serum supplementation can be reduced by 50–90% with no change in cellular proliferation or morphology. Many cell lines do not need to be domesticated to use this medium. Cells successfully cultured in Advanced DMEM/F12, with no adaptation, include Vero, LX-2, KGN, C8-D1A, HeLa, 4T1, NIH-3T3, KLE, ARPE-19, JHH-7, NCI-H2009, etc.

Advanced DMEM/F12 medium can achieve the serum-reduced effect due to the addition of the following ingredients: ethanolamine, glutathione, ascorbic acid, insulin, transferrin, bovine serum albumin and various trace elements (sodium selenite, ammonium metavanadate, copper sulfate and manganese chloride).

### Prepare Complete Media

Advanced DMEM/F12 medium require supplementation with 1-5% fetal bovine serum (FBS) and 1% L-alanyl-L-glutamine solution (GlutaMAX) (Cat. No.: PB180419) to maintain normal cell growth.

To prepare 1 L Advanced Reduced Serum Complete Media:

1. Aseptically add 20 mL GlutaMAX (200 mM).
2. Aseptically add 10-50 mL FBS.

**Note:** Optimize the FBS concentration for each cell line to obtain maximum serum reduction.

3. Add antibiotics, if required. Cellular growth may be impeded by the addition of antibiotics. We recommend reducing the amount of antibiotic by the same percentage that serum supplementation is reduced.

## Culture Use Conditions

**Temperature:** 37°C

**Incubator atmosphere:** Humidified atmosphere of 5-10% CO<sub>2</sub> in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Adapt cell lines to reduced serum media

For most cell lines, no adaptation is necessary (see **Direct adaptation**) to attain at least a 50% reduction in serum supplementation levels. If suboptimal cell growth characteristics (e.g., growth rate, morphology, or secondary metabolite production levels) are observed or additional serum reduction is desired use the **sequential adaptation** procedure. Successful adaptation will depend on the particular cell line and the culture conditions employed. We recommend maintaining backup cultures in the original medium until achieving success with the new medium.

**Note:** For best results cell viability should be  $\geq 90\%$  and growth rate be in mid-logarithmic phase prior to adaptation.

### Direct adaptation

1. Subculture cells grown in conventional medium with 5–10% FBS into the appropriate (see **Table 1**) prewarmed complete Advanced Media.
2. Monitor cell growth and subculture following your normal protocol using the appropriate (see **Table 1**) prewarmed complete Advanced Media.
3. Cell cultures are considered to be adapted after 3–5 passages of consistent growth.

**Note:** If suboptimal performance is observed using the direct adaptation method over 3-5 passages, use the sequential adaptation method.

### Sequential adaptation

1. Subculture cells grown in conventional medium with 5-10% FBS into a 25:75 ratio of the appropriate (see **Table 1**) prewarmed complete Advanced Media to the original media.
2. Monitor cell growth and subculture following your normal protocol into stepwise increasing ratios of new media to the original media (50:50, followed by 75:25, then 90:10). Multiple passages at each step may be needed.
3. Subculture cells into 100% prewarmed complete Advanced Reduced Serum Media, and continue to monitor and passage cells until consistent growth is achieved. After several passages of consistent growth and viability in 100% complete Advanced Reduced Serum Media the culture is considered to be adapted.

**Table 1** Recommended Serum Levels. For use in Advanced Serum Reduced Media:

Cell line	% FBS
Vero	5%
LX-2	5%
KGN	5%
UMNSAH/DF-1	5%
C8-D1A	5%
Hela	5%
EA.hy926	5%
CRFK	2-5%
U-2 OS	2-5%
MDA-MB-468	2-5%
JEG-3	5%
SiHa	2-5%
KLE	5%
ARPE-19	2-5%
4T1	2-5%
NIH-3T3	2-5%
BRL	2-5%
JHH-7	5%
NCI-H2009	5%

(For cells outside the list, please refer to the above method for testing and optimization)

## Notes

1. Not all cells are suitable for reduced serum culture. Be sure to test the effect in a small amount before replacing it. If necessary, the FBS concentration should be optimized for each cell line to obtain maximum serum reduction.
2. This product has been filtered and sterilized. Pay attention to aseptic operation to avoid contamination.
3. To maintain the best use of this product, do not perform freeze-thaw treatment.
4. For research use or further manufacturing. Not for diagnostic use or direct administration into humans or animals.