

Advanced RPMI 1640 and D-MEM/F-12

P R O D U C T N E W S

- *Reduce FBS supplementation 50-90% with no loss of performance*
- *Extend the life of your serum lot*
- *Decrease variability caused by lot-to-lot changes of serum*
- *Save time and money*

Traditionally, researchers using mammalian cell culture must add 5-20% animal serum to standard media formulations in order to cultivate their cells. Gibco® Advanced RPMI 1640 and D-MEM/F-12 are standard basal formulations enriched with additional nutrients that are normal constituents of serum, enabling reduction in Foetal Bovine Serum (FBS) supplementation by 50-90%, with little or no change in cell growth, promotion, morphology, or function (Figure 1).

Reduce serum concentration

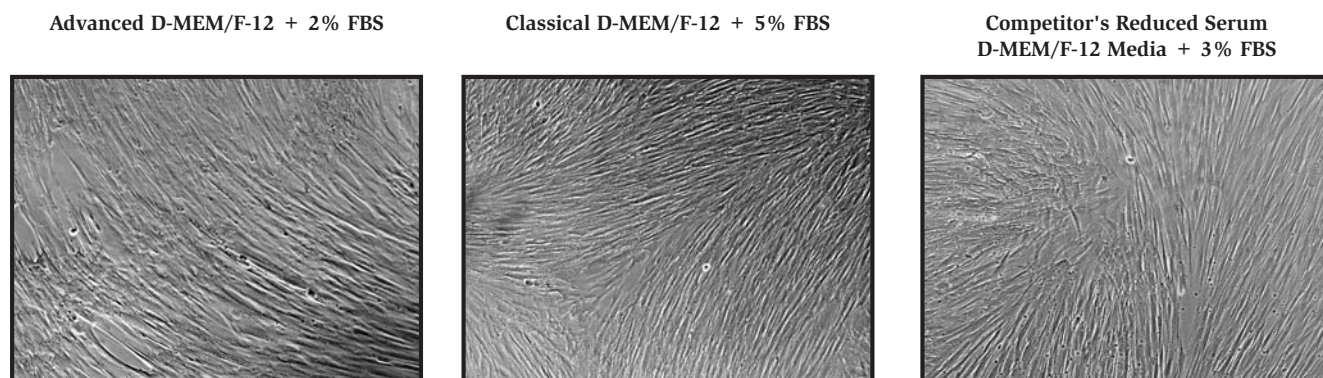
Each Advanced Medium is the standard published basal formulation further supplemented with non-essen-

tial amino acids (NEAA) and sodium pyruvate. The following ingredients have also been added to reduce serum concentration: ethanolamine, glutathione (reduced), ascorbic acid phosphate, recombinant insulin, human (holo) transferrin, AlbuMAX® (a lipid-rich bovine serum albumin), and the trace element salts sodium selenite, ammonium metavanadate, cupric sulfate and manganous chloride. For complete formula information, please visit www.invitrogen.com.

For most applications, no weaning or adaptation procedures are necessary to reduce serum supplementation by at least 50% in a wide variety of cell lines. Conversion to Advanced

Media is obtained by simply centrifuging cells, decanting the supernatant, and resuspending cells in the reduced-serum supplemented medium. Although the recommended concentration of serum with Advanced Media is 0.5-5% (Figures 2 and 3), the concentration must be adjusted for each individual cell line to obtain optimal results. The serum concentration reductions achieved will vary with the cell line and medium used. To obtain a FBS reduction greater than the recommended level, we suggest to titer your FBS concentration to determine maximum reduction. In some cases, additional serum reduction may be obtained by minimal weaning.

Figure 1 – Cell growth and morphology comparison



Advanced D-MEM/F-12 supplemented with 4 mM L-Glutamine and 2% FBS was compared to classical D-MEM/F-12 supplemented with 4 mM L-Glutamine and 5% FBS and the competitor's reduced serum D-MEM/F-12 media supplemented with 4 mM L-Glutamine and 3% FBS (100X magnification). MRC-5 (diploid human lung) cells were plated, without pre-adaptation, to Advanced D-MEM/F-12, Classical D-MEM/F-12 (Gibco® Cat. no. 11320), or a competitor's reduced serum D-MEM/F-12 media at 4.0×10^4 per well in 24-well plates (1 ml per well), incubated at 37°C with 5% CO₂ and 95% air over a 4-day passage cycle. Cell growth and morphology for Advanced D-MEM/F-12 was comparable to or better than the alternative conditions.

Extend the life of serum lots, decrease variability

While reducing the need for serum, Advanced Media can also save time, labour, and money usually spent in qualifying new lots, since decreasing the serum concentration increases the lifespan of your serum lot. Additionally, where there are fewer lot-to-lot changes, there are fewer chances of experimental variability and reduced risk of interference or variability from undefined proteins or other serum constituents.

Additional tips

Advanced RPMI 1640 and D-MEM/F-12 are provided in a 1X liquid format and need only the addition of L-Glutamine

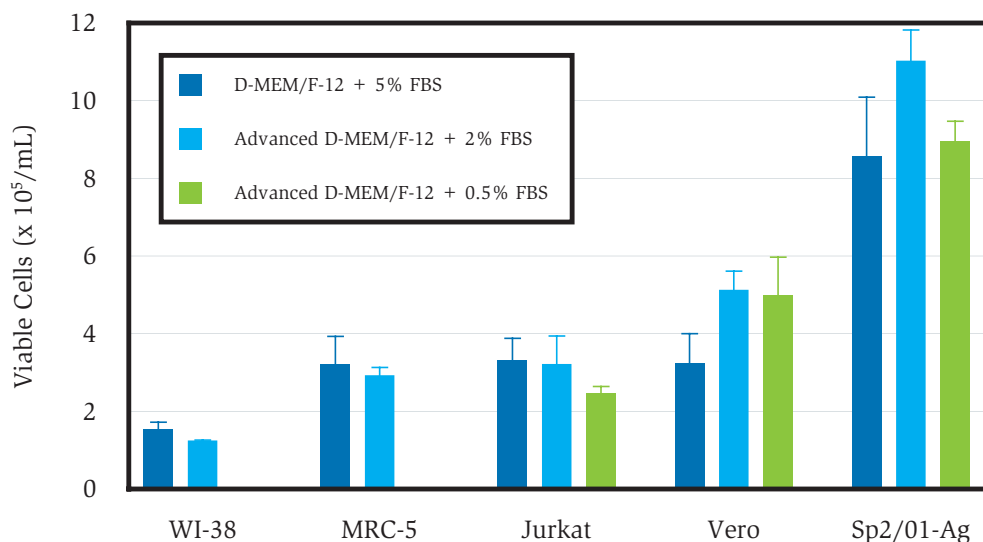
or GlutaMAX™-I supplement and the appropriate reduced amount of serum. Tables 1 and 2 provides recommended seed density and percent FBS for various cell types. If you are using antibiotics to control contamination, it is important to reduce the quantity to correspond with the reduced amount of serum. For example, if you reduce serum by half, then reduce antibiotics by half; if you reduce the serum 10-fold, then reduce the antibiotics 10-fold. No reduction is required for selective antibiotics.

Table 1 – Advanced D-MEM/F-12 applications

Cell Types	Recommended Seed Density (viable cells/25 cm ²)	% FBS in Advanced D-MEM/F-12
Vero	1.0 x 10 ⁵	0.5 – 2
Jurkat	5.0 x 10 ⁵	1 – 2
Sp2/01-Ag	1.5 x 10 ⁵	0.5
MRC-5	5.0 x 10 ⁵	2
WI-38*	5.0 x 10 ⁵	2

*For the best results, we recommend using Advanced MEM (Gibco® Cat. no. 12492-013) with the WI-38 cell line.

Figure 2 – Cell line growth: Classical vs. Advanced D-MEM/F-12



D-MEM/F-12 (Gibco® Cat. no. 11320) supplemented with 4 mM L-Glutamine and 5% FBS was compared to Advanced D-MEM/F-12 supplemented with 4 mM L-Glutamine and 0.5-2% FBS (cell line dependent). The cell lines were plated at recommended seeding densities identified in Table 1. Each cell line was cultured over a three-passage period; the first two passages were conducted in 25 cm² flasks and the final passage in triplicate wells of a 24-well tissue culture plate. All flasks and plates were incubated at 37°C with 5% CO₂ in air. Sub-passaging was conducted at 3-4 day scheduled intervals. Data depicted in the graph above represents the mean of triplicate (Cedex) measures conducted on day five of the third passage.

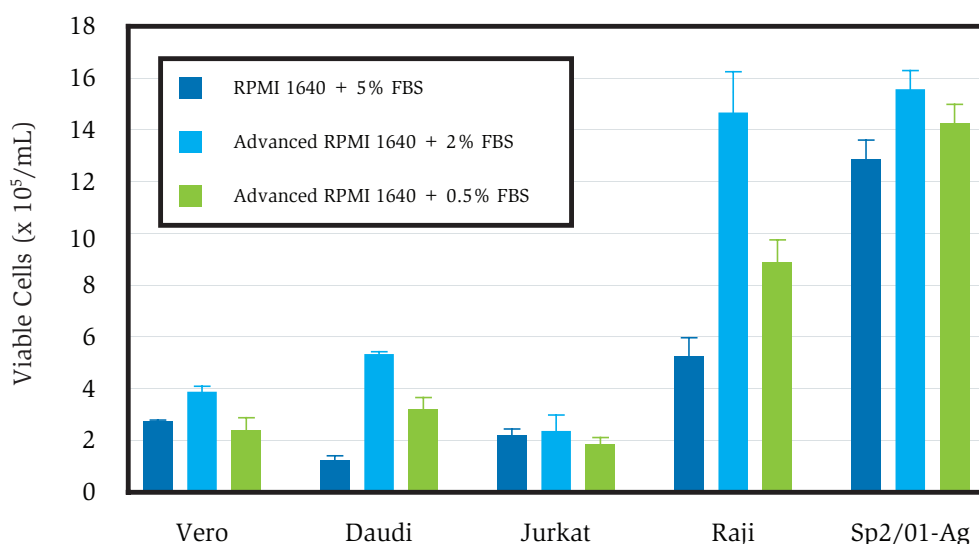
Request a sample

To try any of the Advanced Media formulations, contact your local Invitrogen representative.

Table 2 – Advanced RPMI 1640 applications

Cell Types	Recommended Seed Density (viable cells/25 cm ²)	% FBS in Advanced RPMI 1640
Vero	1.0 x 10 ⁵	0.5 - 2
Jurkat	5.0 x 10 ⁵	0.5 - 2
Sp2/01-Ag	1.5 x 10 ⁵	0.5
Raji	1.5 x 10 ⁶	0.5
Daudi	1.0 x 10 ⁶	0.5

Figure 3 – Cell line growth: Classical vs. Advanced RPMI 1640



RPMI 1640 (Gibco® Cat. no. 21875) supplemented with 4 mM L-Glutamine and 5% FBS was compared to Advanced RPMI 1640 supplemented with 4 mM L-Glutamine and 0.5-2% FBS (cell line dependent). The cell lines were plated at recommended seeding densities identified in Table 2. Each cell line was cultured over a three-passage period; the first two passages were conducted in 25 cm² flasks and the final passage in triplicate wells of a 24-well tissue culture plate. All flasks and plates were incubated at 37°C with 5% CO₂ in air. Sub-passaging was conducted at 3-4 day scheduled intervals. Data depicted in the graph above represents the mean of triplicate (Cedex) measures conducted on day 5 of the third passage.

Get what you need for better cell culture!

Ordering information

Description	Size	Cat. no.
Advanced D-MEM/F-12 (1X), liquid <i>Contains 3,151 mg/L D-glucose, 110 mg/L sodium pyruvate, and non-essential amino acids, but no L-Glutamine.</i>	500 ml 10 x 500 ml•	12634-010 12634-028
Advanced RPMI 1640 (1X), liquid <i>Contains 2,000 mg/L D-glucose, 110 mg/L sodium pyruvate, and non-essential amino acids, but no L-Glutamine.</i>	500 ml 10 x 500 ml•	12633-012 12633-020

Related Products

Advanced D-MEM (1X), liquid <i>Contains 4,500 mg/L D-glucose, 110 mg/L sodium pyruvate, and non-essential amino acids, but no L-Glutamine.</i>	500 ml 10 x 500 ml•	12491-015 12491-023
Advanced MEM (1X), liquid <i>Contains 110 mg/L sodium pyruvate, and non-essential amino acids, but no L-Glutamine.</i>	500 ml 10 x 500 ml•	12492-013 12492-021

Nutritional supplements

Foetal Bovine Sera and other Sera	See Chapter 2 of the 2004 Gibco® catalogue	
GlutaMAX™-I Supplement <i>Contains the dipeptide L-alanyl-L-Glutamine; can be directly substituted for L-Glutamine.</i>	100 ml	35050-038
L-Glutamine-200 mM (100X), liquid	100 ml	25030-024

Antibiotics

Gentamicin Reagent Solution (10 mg/ml)	10 ml	15710-049
Gentamicin Reagent Solution (50 mg/ml)	10 ml	15750-037
Penicillin-Streptomycin (100X) <i>Contains 10,000 units of penicillin (base) and 10,000 µg of streptomycin (base)/ml utilizing penicillin G (sodium salt) and streptomycin sulfate in 0.85% saline.</i>	20 ml 100 ml	15140-148 15140-122

Wash buffers

Balanced Salt Solutions	See Chapter 3 of the 2004 Gibco® catalogue	
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Cell dissociation

TrypLE™ Express, with Phenol Red	100 ml	12605-010
	500 ml	12605-028
TrypLE™ Express, without Phenol Red	100 ml	12604-013
	500 ml	12604-021
Trypsin-EDTA (0.05% Trypsin, EDTA•4Na)	100 ml	25300-054
Trypsin-EDTA (0.25% Trypsin, EDTA•4Na)	100 ml	25200-056

- EasyPack™



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