Advanced RPMI 1640 and D-MEM/F-12

PRODUCT NEWS

- *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 Fetal Bovine Serum (FBS) supplementation by 50-90%, with little or no change in cell growth, promotion, morphology, or function (Figure 1).

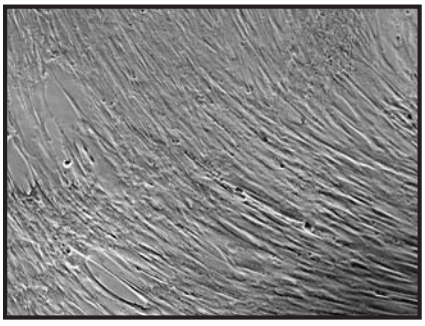
Reduce serum concentration

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

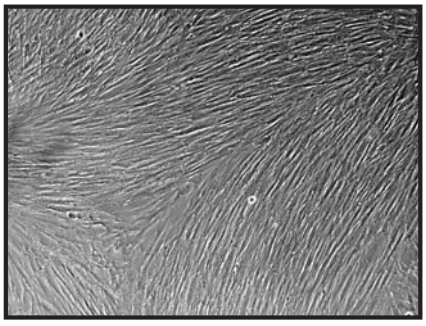
For most applications, no weaning or adaptation procedures are necessary to reduce serum supplementation by at least 50% in a wide variety of cell lines. Conversion to Advanced Media is obtained by simply centrifuging cells, decanting the supernatant, and resuspending cells in the reduced-serum supplemented 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

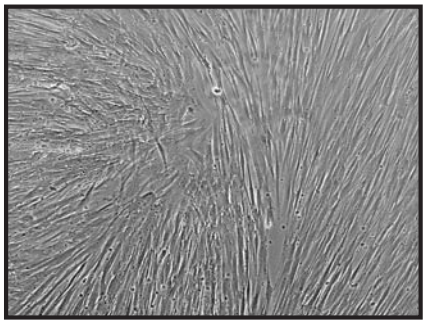
Advanced D-MEM/F-12 + 2% FBS



Classical D-MEM/F-12 + 5% FBS



Competitor's Reduced Serum D-MEM/F-12 Media + 3% FBS



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. 11320-033), or a competitor's reduced serum D-MEM/F-12 media at 4.0 x 10^4 per well in 24-well plates (1 ml per well), incubated at 37°C with 5% CO_2 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.

Gibco®
invitrogen cell culture

Extend the life of serum lots, decrease variability

While reducing the need for serum, Advanced Media can also save time, labor, 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

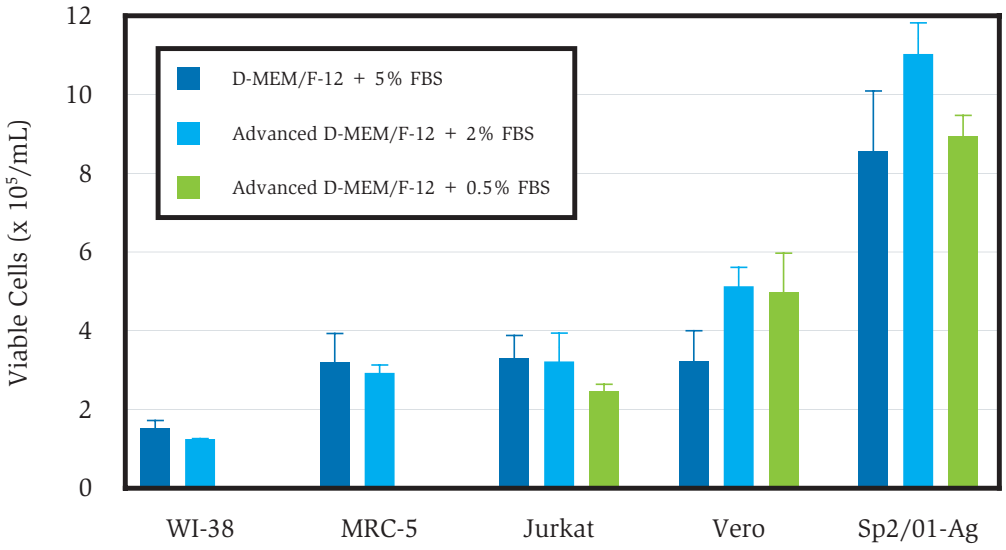
Advanced RPMI 1640 and D-MEM/F-12 are provided in a 1X liquid format and need only the addition of L-glutamine or GlutaMAX™-I supplement and the appropriate reduced amount of serum. Tables 1 and 2 provides recommended seed density and percent FBS for various cell types. If you are using antibiotics to control contamination, it is important to reduce the quantity to correspond with the reduced

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	1.0×10^5	0.5 – 2
Jurkat	5.0×10^5	1 – 2
Sp2/01-Ag	1.5×10^5	0.5
MRC-5	5.0×10^5	2
WI-38*	5.0×10^5	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. 11320-033) 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 cm^2 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% CO_2 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.

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.

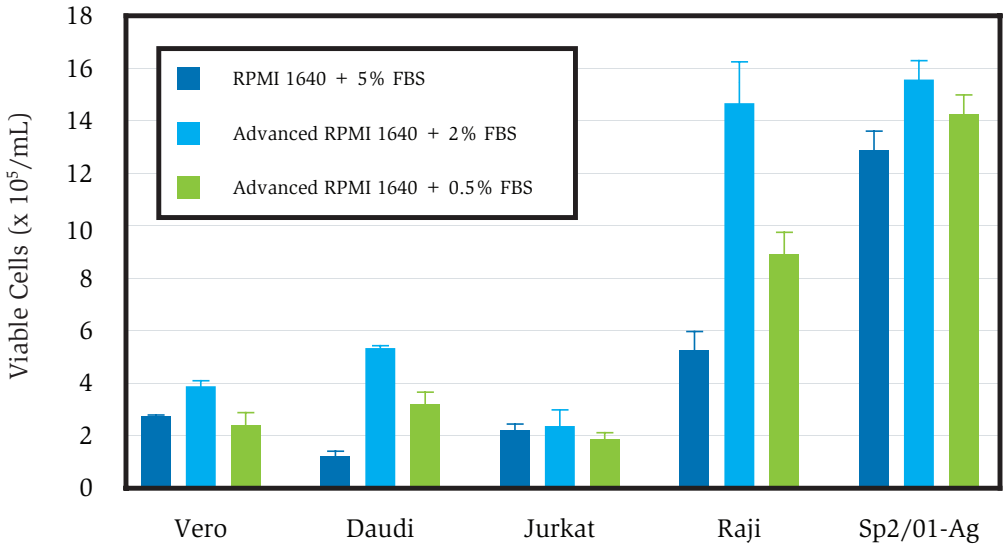
Request a sample

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. 11875-093) 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 cm^2 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% CO_2 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.

Ordering information

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

Related Products

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

Nutritional supplements

Fetal Bovine Sera and other Sera	See Chapter 2 of the 2004 Gibco® catalog	
GlutaMAX™-I Supplement *Contains the dipeptide L-alanyl-L-glutamine; can be directly substituted for L-glutamine.*	100 ml	35050-061
L-Glutamine-200 mM (100X), liquid	100 ml	25030-081
L-Glutamine	100 g	21051-024

Antibiotics

Gentamicin Reagent Solution (10 mg/ml)	10 ml	15710-064
Gentamicin Reagent Solution (50 mg/ml)	10 ml	15750-060
Penicillin-Streptomycin (100X) *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.*	20 ml	15140-148

Wash buffers

Balanced Salt Solutions	See Chapter 3 of the 2004 Gibco® catalog	

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

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Corporate headquarters:
1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com

European headquarters:
Invitrogen Ltd • Inchinnan Business Park • 3 Fountain Drive • Paisley PA4 9RF, UK • Tel: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com

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