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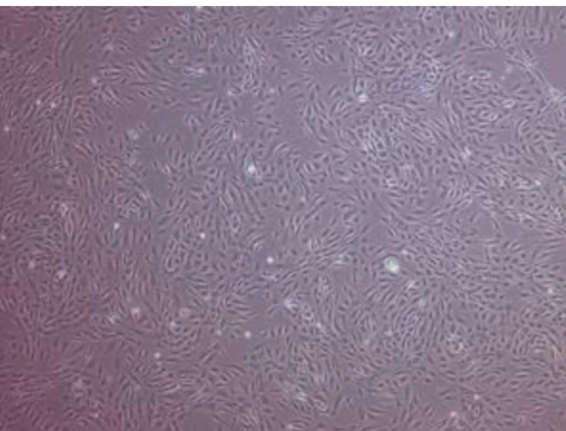
Instructions for use for XerumFree™ XF212 Medium Supplement



Instructions For Use - XerumFree™ XF212 Medium Supplement. Fully defined, animal-component free cell culture supplement.

Performing cell culture without serum can be challenging. However, the rewards do largely recompense the efforts, and re-discovering the basics of cell culture develops quickly into a passion. The intention of this paper is to guide the user to a smooth transition to serum-free conditions and to avoid all inadequate or inappropriate efforts.

Ideally, the transition to serum-free conditions should be carried out over several passages to gradually select cells that can grow under serum-free conditions. However, direct adaptation to serum-free environments may also work out successfully, provided that all crucial aspects are addressed properly.

Regardless of the method used, key concerns include the growth state of the cellular inoculum, cell seeding density, sub-cultivation techniques, and biophysical attributes of the cell culture system.

XerumFree™ serum replacement has been designed so as to be used in the same way as conventional cell culture sera, as a medium supplement.

IMPORTANT NOTES BEFORE USING Concentrated XerumFree™ XF212:

- XF212 is a cell culture medium additive replacing serum, so it is not a final medium.
- XF212 must be added to a basal cell culture medium (e.g. IMDM, DMEM-F12 or any other basal medium of choice).
- XF212 does not contain growth factors like cytokines, hormones etc. Therefore also no insulin*.

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1 Preparing XF212

1.1 GENERAL PREPARATION OF THE SERUM-FREE CELL CULTURE MEDIUM

Gently shake the bottle of XerumFree™ shortly before use.

Add XerumFree™ to your preferred basal medium at the same concentration compared to serum (e.g. 10%).

Do not add any antibiotics at this stage. In fact, antibiotics like many compounds bind to the plasma proteins of serum, in particular to the albumin fraction. Thus, the same concentration of antibiotics will exhibit a much higher biological activity in serum- and albumin-free conditions and this increased activity may have deleterious impacts on cell growth.

In case 'antibiotic-free culture' is deemed unworkable, the use of gentamycin is suggested at the concentration of 50 mg/l.

* *Some users prefer or need to grow their cells free of insulin. In case of XerumFree™ the decision to grow cells with or without insulin (and/or other growth factors) is yours. If insulin is needed for cell growth and performance we advise to add recombinant insulin in a concentration of 1.25 mg/l final cell culture medium.*

1.2 GENERAL ADAPTION METHODS TO SERUM-FREE CONDITIONS

There are basically two approaches to adapt cells to growth in serum-free environment:

1.2.1 Direct Adaptation
Which is carried out by a direct transfer of the cells from the serum-containing medium into the serum-free medium.

1.2.2 Sequential Adaptation or Weaning Method
Pass the cells from the original serum containing medium sequentially through the following phases where each step halves the serum-supplemented media, thus increasing the serum-free media to aproximately below values:

Phase 1:
50% XerumFree-supplemented medium /
50% Serum-supplemented medium

Phase 2:
75% XerumFree-supplemented medium /
25% Serum-supplemented medium

Phase 3:
87.5% XerumFree-supplemented medium /
12.5% Serum-supplemented medium

Phase 4:
93.75% XerumFree-supplemented medium /
6.25% Serum-supplemented medium

Phase 5:
96.88% XerumFree-supplemented medium /
3.12% Serum-supplemented medium

Phase 6:
98.44% XerumFree-supplemented medium /
1.56% Serum-supplemented medium

Phase 7:
100% XerumFree-supplemented medium

In case of reduced growth go back one step and continue after growth is established again.

Cell cultures may consist of cell lines (adherent or suspension growth) or primary cultures. Moreover, from a functional point of view, cell types may be differentiated to various degrees or exhibit undifferentiated characteristics, as in the case of stem cell preparations. In each case, the adaptation protocol has to take into account the specific requirements of the cell type in order to guarantee the best chances for success.

2 Use of XF212

2.1 CELL LINES

The following protocols are valid for normal (diploid, limited lifespan) or transformed or immortalized cell lines (with indefinite lifecycle).

2.1.1 Anchorage-dependent Cell Lines

Critical success factors:

- coating of the cell culture support for optimal cell attachment
- minimize action of trypsin
- choice of the antibiotic system

Experimental Steps:

A) Coat the cell culture surface with an adequate cell-attachment factor by using:
- a commercial coating kit such as Pronectin™ F, MapTRIX™ or equivalent.
- a Fibronectin or Poly-L-Lysine coating, or
- a little FBS (e.g, 500 µl for a T25 flask) with overnight incubation at 37°C, followed by two washes with PBS or fresh medium.
- ready to use plastics that provide an improved attachment of adherent cells.

B) Dissociate the cell monolayer
- The use of standard trypsin preparations can become somewhat problematic in the absence of serum, which contains trypsin inhibitors. It is therefore important to minimize the proteolytic activity of residual trypsin in serum-free conditions in order to avoid irreversible damage to the cells. This can be best achieved by the use of trypsin inhibitors (e.g. from soybean) or by employing a non-mammalian dissociation reagent such as Accutase™ which does not require inactivation or removal during passaging. Alternatively an additional wash step of the cell pellet will remove most of the remaining trypsin. However, this procedure implies an extra centrifugation step that can be damaging for some cell types.
- Our preference: forget about trypsin at all and use Accutase™ or Detachin™ to dissociate the cell monolayer; these cell detach ment solutions have been developed to meet the most demanding requirements for gentle and effective detachment of adherent cells; cell membranes and surface epitopes will not be harmed and the structural and functional quality of the surface proteins remain intact.

C) Seed cells at 20,000 cells per cm^2 in complete medium as prepared under point 1.
- It is important to observe a high seeding density during the first steps of the adaptation process. Cells normally secrete a host of factors into the culture medium that control cell attachment, growth and proliferation. However, during the seeding step these factors are absent in the fresh serum-free medium and a critical level of cell density is essential to induce an immediate and sufficient production of these autocrine/paracrine factors.

D) Incubate and maintain the cell cultures at 37°C until they reach 80-90% confluency.
- During this period change 75% of the medium every 2-3 days. Do not discard the spent medium. Instead harvest the conditioned medium, sterile filter and put aside at 4°C for use in the next steps. If the cells seem stalled at any point, allow them more time to adapt to their new serum-free environment.

E) When near confluency is reached, split the cells at a 1:2 or 1:3 ratio.
- For this second passage in XerumFree™ a coating is not required but use of conditioned medium is strongly suggested - this medium fraction contains indeed the autocrine factors that regulate attachment, spreading, growth and proliferation. Seed cells in a mixture consisting of 75% fresh medium + 25% conditioned medium, collected during the previous passage. Continue supplying cells with 75% fresh medium every 2-3 days and collect the conditioned medium as under d) above.

F) Repeat step E) until the cells exhibit growth dynamics comparable to their former growth in serum-supplemented medium.
- At that point the cell line can be considered fully adapted. This may take up to a total of 4-6 passages.

G) From this point on, antibiotics may be added to the culture medium.
- We advice the use of the large-spectrum antibiotic gentamycin; this antibiotic has a much reduced cytotoxicity as compared to the standard Penicillin/Streptomycin cocktails. The suggested concentration of use of gentamycin is 50 mg/l.

H) Once adapted, the original split ratio (in serum-supplemented conditions) may be applied.

2.1.2 Anchorage-independent Cell Lines

The following protocol is adjusted for cell lines that grow already in suspension. For the adaptation of adherent cells to XerumFree™ suspension growth, please see Technical Note 'Adaptation of Cells From Monolayer to Serum-free Suspension Culture'.

Critical success factor:
Choice of the antibiotic system

Experimental Steps

A) **When cell densities of 3-5 x 10^6 cells/ml are reached (depending on the cell line) start switching to XerumFree™ supplemented medium.** Harvest the cell suspension, take out a small aliquot for cell counting and centrifuge the whole suspension at 200 g for 5 minutes.

B) **Perform a cell count.**

C) **Resuspend the cell pellet in Xerum-Free™ supplemented medium at a density of 10^6 cells/ml.** It is important to observe a high seeding density during the first steps of the adaptation process. Cells normally secrete a host of factors into the culture medium that control cell growth and proliferation. However, during the seeding step these factors are absent in the fresh serum-free medium and a critical level of cell density is essential to induce an immediate and sufficient production of these autocrine/paracrine factors.

D) **Incubate and maintain the cell cultures at 37°C until they reach a density of approximately 3-5 x 10^6 cells/ml.**

E) **Split the suspension cultures at a 1:3 or 1:4 ratio, by adding the appropriate volume of fresh medium (e.g. 25 ml of cell suspension + 75 ml XerumFree™ supplemented medium, to be dispatched into 4 separate culture vessels)**

F) **Repeat step E) until the culture exhibits growth dynamics as originally in serum-supplemented medium.** From then on, the cell line can be considered fully adapted and may be split at the original ratios during serum-supplemented culture.

G) **From this point on, antibiotics may be added to the culture medium.** We suggest the use of Gentamycin at the concentration of 50 mg/liter; this antibiotic has a much lower cytotoxicity as compared to the standard Penicillin/Streptomycin cocktails.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85