

## ISF-1 Medium: Serum-Free Media for Hybridoma Culture

Biochrom AG Information

ISF-1 is a serum-free, chemically defined optimized medium for hybridoma growth and monoclonal antibody production. With serum-free medium, cell culture is a defined process that provides greater consistency between experiments. Further advantages are batch to batch conformity, consistency in process and freedom from concern regarding mycoplasma and other contaminants. Using media without serum also makes it easier to purify a desired protein product from a culture after the cells secrete it into the supernatant, e.g. monoclonal antibodies produced in serum-free media will not be contaminated with unspecific bovine serum antibodies.

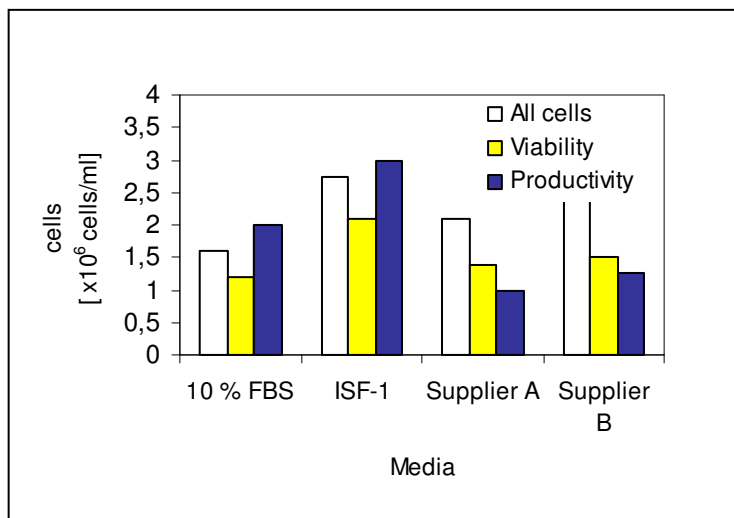
ISF-1 serum-free media supports growth and monoclonal antibody production of mouse hybridoma cell lines. ISF-1 does not contain exotic growth factors and is therefore cost efficient. In comparison to other commercially available serum-free media ISF-1 reveals superior performance with regard to cell growth, viability and antibody productivity (see figure).

### Medium-Characteristic

ISF-1 has been manufactured with stable glutamine. ISF-1 contains a surfactant. Therefore no additional supplement is needed for agitated suspension culture.

ISF-1 works well for a variety of hybridoma systems, but will not grow cholesterol dependent cell lines (e.g. NS0 and derivatives) without further supplementation. Addition of a lipoprotein preparation or other source of cholesterol will be required for these cell lines.

Addition of antibiotics should not be used as a substitute for proper sterile technique. In most instances, antibiotics are neither necessary nor advised. In very rare cases the addition of antibiotics (e.g. penicillin/streptomycin) will reduce the productivity of hybridoma cell lines. However, in those instances where antibiotics are desired, most general antibiotics are compatible with ISF-1 including penicillin/streptomycin, gentamycin, puromycin or fungizone.



**Abb.: Influence of different media on cell growth, viability and productivity**

Hybridoma cells were cultivated in T-flasks. After adaptation of the cells to the different media, cell growth, viability and productivity were measured after one week of cultivation. Productivity is shown in comparison to 10 % FBS containing media.

## Instructions For Use

Physical Conditions: 37 °C ± 0.5 °C in a humidified atmosphere of 5 % CO<sub>2</sub> in air. Caps of flasks should be loosened to permit gas exchange. Cultures may be grown in stationary culture (T-flask) or agitated suspension culture (spinner flasks). Adequate head space should be provided to facilitate gas exchange. Also dialysis systems (e.g. minperm, Sartorius), the „Super-Spinner“ (B.Braun, Melsungen) or stirred tank bioreactors can be used.

Adaptation of cells to serum-free media: A direct adaptation will function in most cases but with ISF-1 media a sequential adaptation protocol can also be used. In both cases, the cells should be in mid-log growth phase with high (> 90 %) viability. Success of the adaptation will depend upon the particular hybridoma cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with ISF-1 has been achieved.

### Direct Adaptation (1:1):

1. Transfer the cells growing in serum supplemented medium to ISF-1 medium which has been prewarmed to 37 °C. Seeding density should correspond to the normal seeding density for the cell line. Incubate the cells at 37 °C in a humidified atmosphere of 5 % CO<sub>2</sub> in air.
2. Subculture the cell line, monitoring cell growth and viability for 4 to 8 passages.
3. If the culture fails to maintain acceptable growth and viability over 4 to 8 passages during direct adaptation, use the sequential adaptation procedure.

### Sequential Adaptation:

1. Inoculate cells at double the normal seeding density in a 75:25 (v/v) mixture of serum supplemented: serum-free medium.
2. After reaching 1 x 10<sup>6</sup> viable cells/ml subculture into a 50:50 (v/v) mixture of serum supplemented: serum-free medium.
3. After reaching 1 x 10<sup>6</sup> viable cells/ml subculture into a 25:75 (v/v) mixture of serum supplemented: serum-free medium.
4. After reaching 1 x 10<sup>6</sup> viable cells/ml subculture into 100 % serum-free medium.

## Quality Control

ISF-1 media is performance tested using a hybridoma cell line. Additional standard evaluations are pH, osmolality and tests for the absence of bacterial and fungal contaminants.

**For research use only.**

**CAUTION: Not intended for human or animal diagnostic or therapeutic uses.**