

Detailed results:**Australian FBS from Biochrom AG suitable for murine embryonic stem cells**

Information from Biochrom AG

Biochrom AG specialises in the distribution of Fetal Bovine Serum (FBS). The FBS offered primarily originates from South America and Australia. It is not only tested for sterility in accordance with the European Pharmacopoeia, but also for bovine viruses. Before being released for sale, FBS is standardly tested for endotoxins, mycoplasma, virus contamination, and antibodies against viruses (BVD-MD, BHV-1, PI3). Additional tests have now successfully shown: Australian FBS from Biochrom AG is suitable for murine embryonic stem cells.

During the tests, stem cells displayed high viability of 85-91 per cent, depending on the respective lot, as well as very good proliferation in the toxicity assay. Please find below all test results in detail.

1 Biochrom AG quality criteria for FBS

Fetal Bovine Serum (FBS) is the most commonly used supplement (2-20 % in medium) in cell culture systems. Where applicable, it is also used for the production of therapeutic proteins. FBS is a highly complex mixture of serum proteins, amino acids, peptides, growth factors, hormones etc. This is why FBS represents a supplement in cell culture that can be used universally.

Biochrom AG's FBS is based on a thorough selection of the respective raw serum. Aseptic serum filling is performed within an area of cleanliness level A with a background environment B in accordance with the supplementary GMP guidelines for the manufacture of sterile pharmaceuticals, annex 1. All sera are tested for a potential contamination with mycoplasma. Sera are only released if the result is negative.

The raw FBS imported from Australia is delivered to Biochrom AG with the cold chain not being interrupted. After being examined and released by the quality control department, the FBS is thawed under standardised conditions, sterile filtered via a multi-stage filter cascade and then filled using different packing units. Biochrom AG's standard lot size is 1,000 l but can vary up to 2,000 l in accordance with customer-specific requirements. Customers in Asia and America are supplied directly from Australia. A certificate of analysis is provided for each lot.

2 Material and methods

- cell line used: muES HM1 wt, passage 32 (murine embryonic mouse stem cell, strain:129/Ola)
- cells derived from blastocysts
- cell culture medium: GMEM, L-glutamine, non-essential amino acids (NEA), Na-pyruvate, b-mercaptoethanol, penicillin/streptomycin, FBS (cat. no. S 0415, lots 0879 L and 1319 L)
- cell culture vessels: gelatine-coated

3 Experiment set-up

The cultivation of the muES cells takes place in culture vessels of 25 cm² and 75 cm². The cells were passaged at a confluence of 90 %, with their viability being determined for a period of 20 days. The experiment starts in passage 32.

Within the framework of the MTT assay, 5,000 and 10,000 cells/well are seeded on a gelatine-coated 96-well cell culture plate. The medium volume is in both cases 200 µl for a culture period of 20 hours. The assay is performed in passage 37 and 39.

table 1: preparation viability of the cultures

passage	culture period (d)	viability (%) control	viability (%) 0879 L	viability (%) 1319 L
34	4	60	73	76
35	6	39	73	71
36	10	82	86	83
37	13	88	84	87
38	16	69	61	88
39	20	89	85	91

4 Results

Those muES cells that have been cultivated using the lots 0879 L and 1319 L display a viability of 85 and 91 per cent after a culture period of 20 days. The control arrangement displayed a comparable value of 89 %.

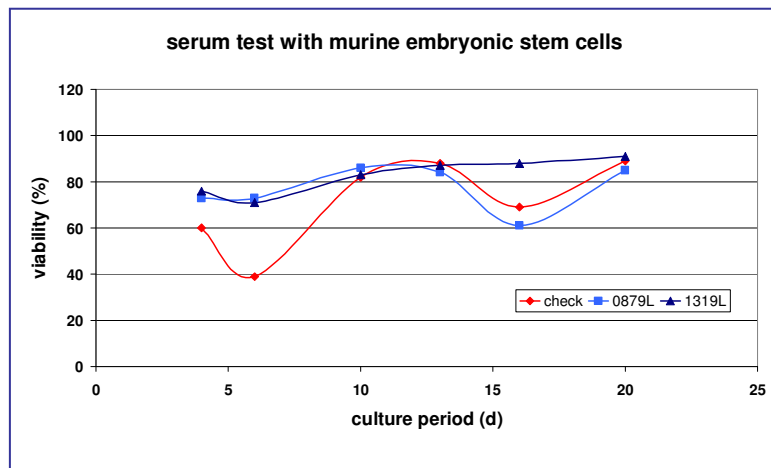


fig. 1: viability control

During the MTT assay, lot 1319 L performed well both in case of low cell density and in case of high cell density. In comparison to the control lot, lot 0879 L is also well suitable for cultivating muES (cf. figures 2 and 3).

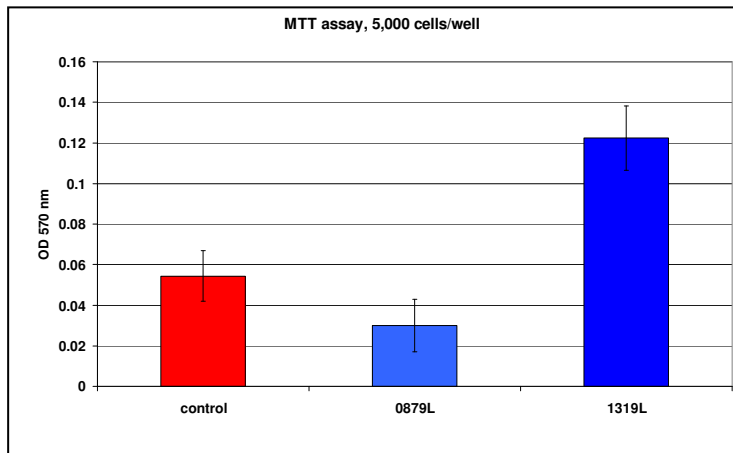


fig. 2: MTT assay with a cell density of 5,000 cells/96 well

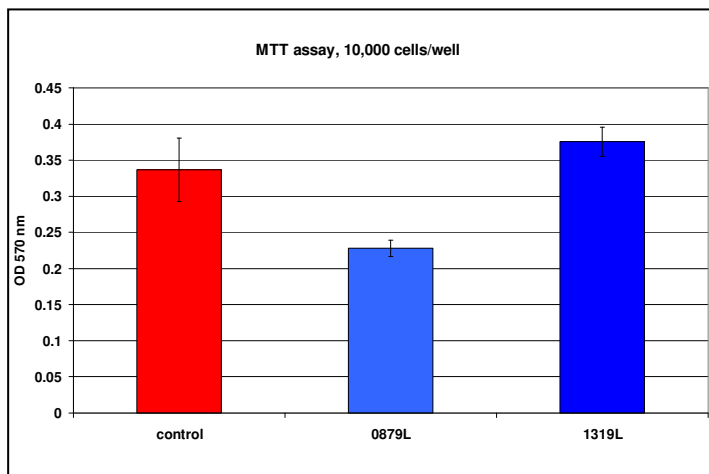


fig. 3: MTT assay with a cell density of 10,000 cells/96 well

5 Summary

- Australian FBS of lots 0879 L and 1319 L is suitable for murine embryonic stem cells.
- The murine embryonic stem cells tested displayed a high viability (85-91 %, depending on the lot).
- The respective muES cells featured very good proliferation (see MTT assay) in these sera.

6 Product details

parameter	Australian FBS
cat. no.	S 0415
unit	500 ml
storage	-20 °C
raw material	serum from Australia
use	suitable for murine embryonic stem cells
note	<ul style="list-style-type: none">➤ for "<i>in vitro</i>" use only➤ with Certificate of Suitability, CoS; (R1-CEP 2001-032)➤ tested for viruses in accordance with EMEA guidelines

- Product information on all sera offered by Biochrom AG:
<http://www.biochrom.de/en/products/sera/>
- Please use the following link to order your free FBS sample:
<http://www.biochrom.de/en/products/sera/sample-order-fbs/>