

# Development of Animal Component-Free Media for the Cryopreservation of Drosophila S2 Cells

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Drosophila Schneider 2 (S2) cells are commonly cryopreserved for long-term storage for use in biological and pharmaceutical product research. While fetal bovine serum (FBS) allows for higher viable cell counts during the freezing and thawing process, the cryoprotectant and other animal component-containing substances require extensive, high-cost testing to ensure that the cell lines and products are not contaminated with adventitious agents. In this study, ten cryopreservation media were tested to determine an animal component-free (ACF) substance(s) capable of replacing FBS. Using ACF substances reduces the cost of research by approximately \$1 million, making the resulting biological product more affordable for the community, and ensures the products' safety for consumers. The freeze media (FM) was combined with S2 cells immediately prior to undergoing cryopreservation, or freezing of cells at -70°C for 24-48 hours and transferring the samples to liquid nitrogen for long-term storage. The viable cell densities (VCD; cells/mL) and viability percentages (% viability) of each cell vial were calculated on day 0 and day 5 post-thaw, during which cells were stored in an incubator. Compared to FM #7 (D5: 1.77E7 VCD, 95.4% viability), FM #26 (D5: 1.82E7 VCD, 97.66% viability) promoted a greater concentration and percentage of viable cells on D5 post-thaw. This study showed that FM #26, majorly composed of sericin from Bombyx mori, is a suitable ACF substitute for FBS, possibly due to the S2 cells' and sericin's matching class, Insecta. Further studies are needed to confirm the results of each freeze media tested.

## Awards Won:

National Anti-Vivisection Society: First Award of \$10,000