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How many times can I freeze and thaw my serum?

HyClone has always recommended that the number of times serum is frozen and thawed be minimized, but we have not recommended a minimum amount and until now we had no data testing the performance of serum after multiple freeze/thaw cycles. We now have data showing no significant difference in the performance of serum that has been frozen and thawed up to 5 times. Thus, our current position is that serum can be frozen and thawed up to 5 times without any loss in productivity. Here we will present this data.

Methods
One lot of Cosmic Calf Serum and one lot of characterized FBS were chosen for this study. Five bottles from each lot were marked 1x thru 5x. Bottle 1x was kept frozen, and bottles 2x thru 5x were thawed at room temperature under a fan. Each bottle was mixed every 20-30 minutes while being thawed. Upon complete thaw, the bottles were immediately refrozen and left overnight at -20ºC. The process was then repeated for bottles 3x thru 5x, and again repeated for 4x and 5x, and finally for 5x.

Growth Study
5 cell lines were used to determine the ability of the conditioned sera to support growth. The cell lines and the basal media used are listed in table 2. All media were supplemented using 10% of the test sera. The attaching cell lines were seeded in triplicate at cell densities of 1.0 x 104 cells/cm² in T-25 flasks and were counted upon reaching 90% confluence. The non-attaching cell lines were seeded in triplicate at cell densities of 8.0 x 103 cells/ml in T-25 flasks and were counted daily beginning on day 3 until cell viability dropped.

Biochemical Analysis
Samples from all serum bottles were sent for biochemical analysis to determine if a detectable change in commonly tested serum components could be determined.

Precipitation Analysis
Samples were also analyzed for precipitation using a turbidimeter and with the naked eye to see if the additional freezing cycles increased the amount of particulate matter.

Results and Discussion:

Figures 1 – 5 show the growth results. In all cases no significant difference in the growth of the cells was observed. The results of the biochemical analysis show that no significant differences were noticed in the components measured. No detectable differences were noticed when the turbidity was measured with a Turbidimeter. There was also no noticeable turbidity when the bottles were observed with the naked eye. Our conclusion thus is that serum can be frozen and thawed up to 5 times without any noticeable affect on the performance or biochemical composition of the serum, and our recommendation now is that it is appropriate to freeze/thaw serum up to 5x.

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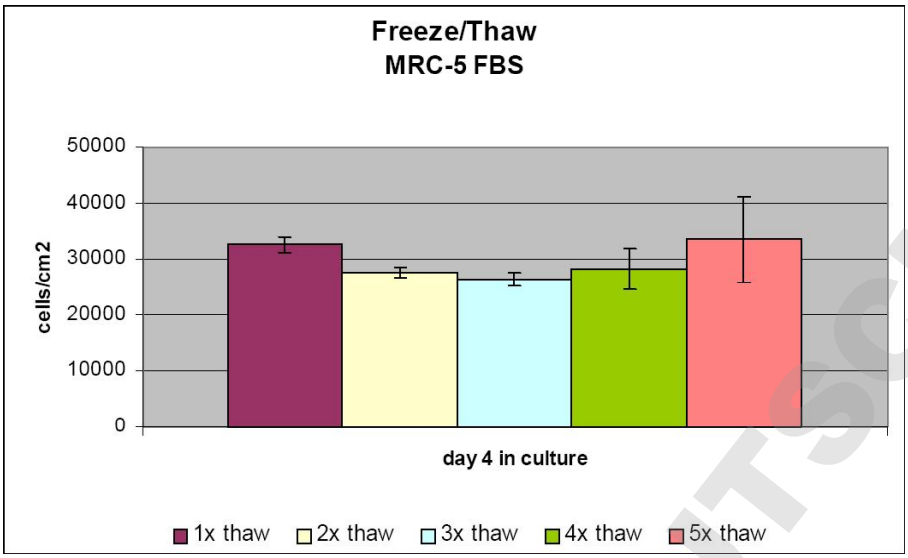

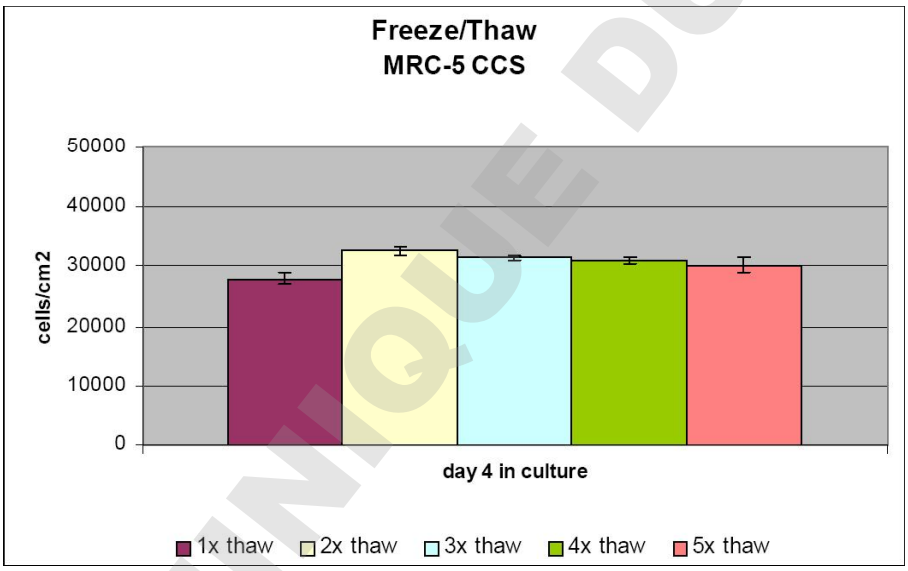

Figure 1: Growth of Vero cells in DME high glucose medium with 10% serum thawed up to 5 times. Cells were initially seeded in triplicate at 1.0 x 10^4cells/cm^2 T-25 flasks and counted upon reaching confluence. Flasks were incubated at 37ºC in a 5% CO_2 atmosphere. Error bars indicate +/- one standard deviation.

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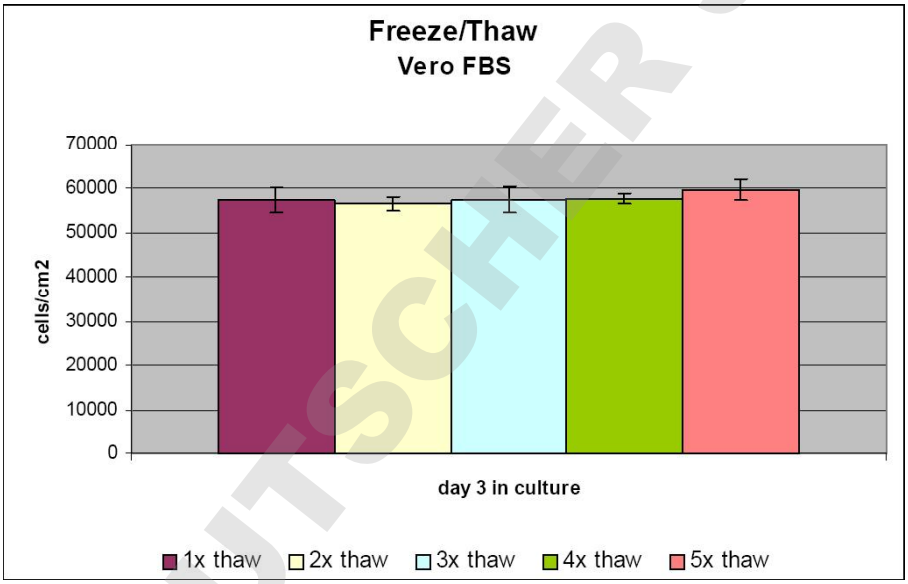
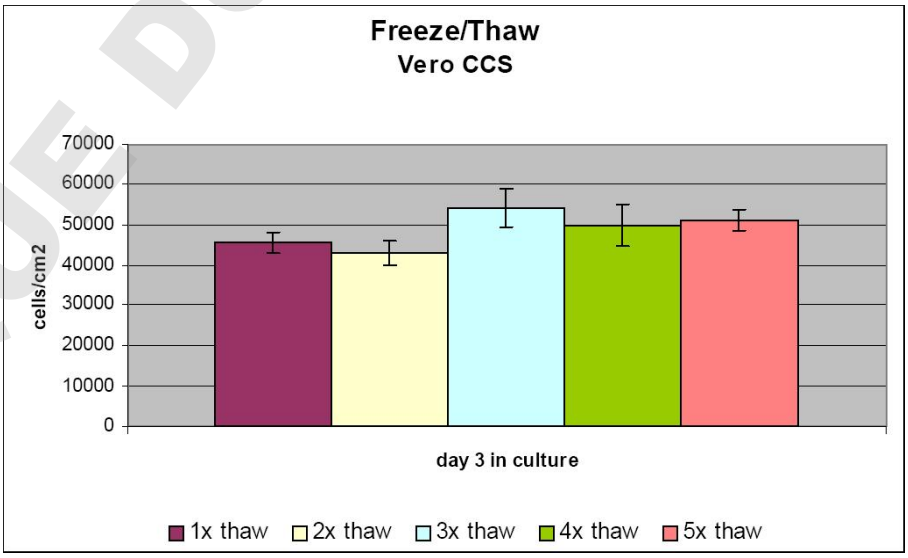


Figure 2: Growth of Vero cells in DME high glucose medium with 10% serum thawed up to 5 times. Cells were initially seeded in triplicate at 1.0 x 10^4cells/cm^2 T-25 flasks and counted upon reaching confluence. Flasks were incubated at 37°C in a 5% CO_2 atmosphere. Error bars indicate +/- one standard deviation.



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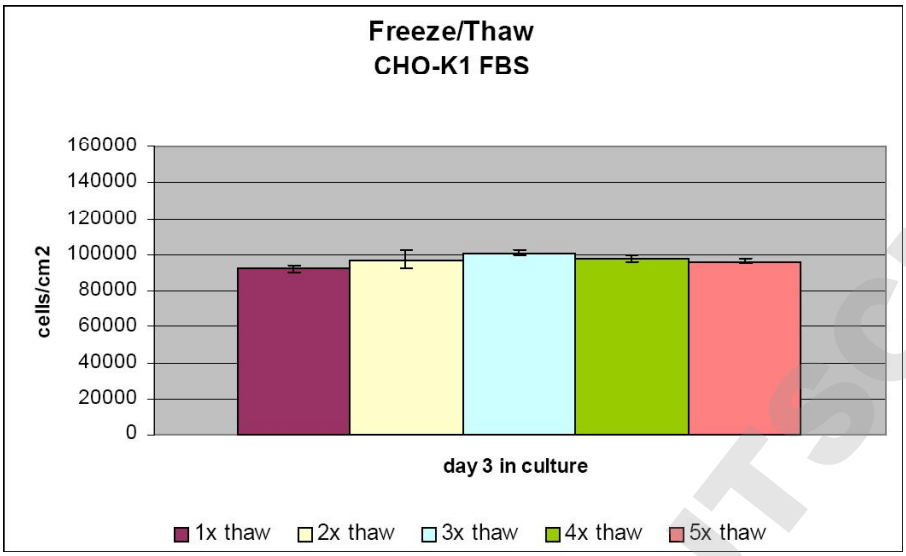

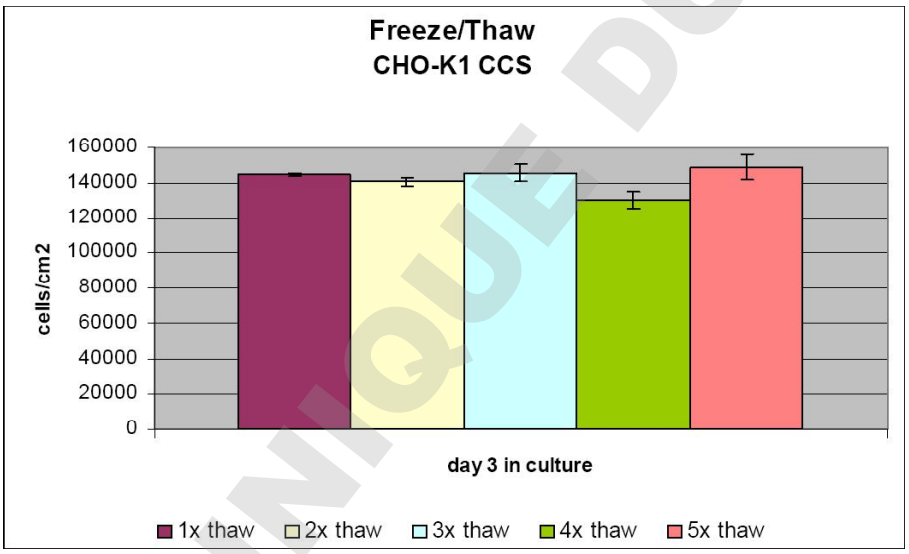

Figure 3: Growth of CHO-K1 cells in Ham's F12 medium with 10% serum thawed up to 5 times. Cells were initially seeded in triplicate at 1.0 x 10^4cells/cm^2 T-25 flasks and counted upon reaching confluence. Flasks were incubated at 37ºC in a 5% CO_2 atmosphere. Error bars indicate +/- one standard deviation.

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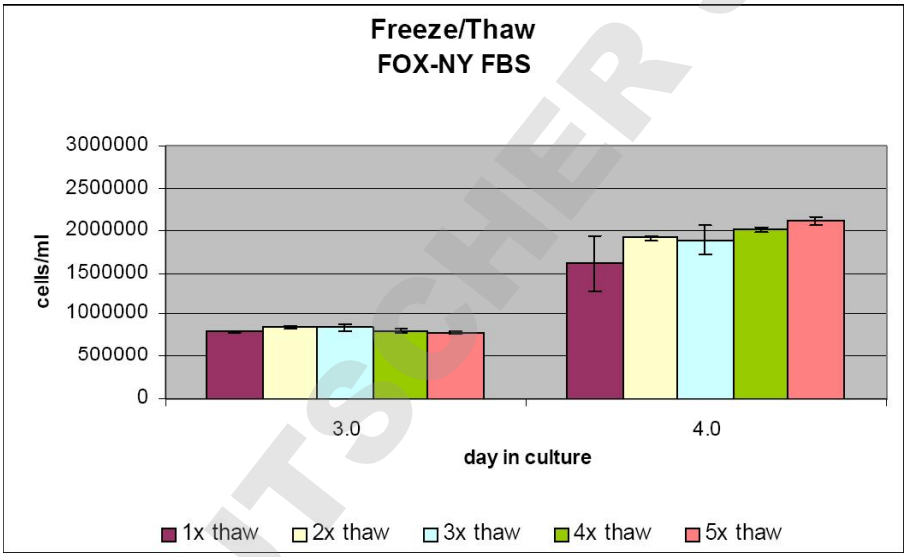

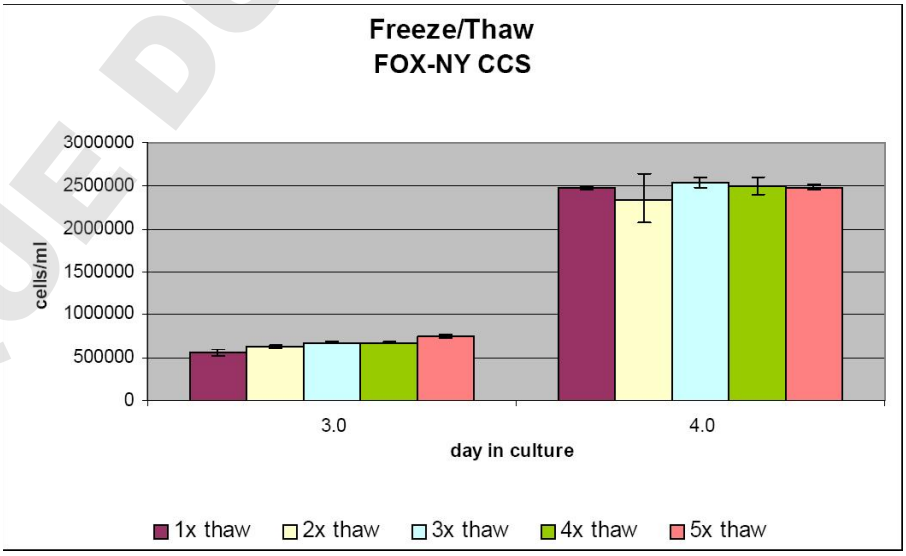

Figure 4: Growth of FOX-NY cells in DME high glucose medium with 10% serum thawed up to 5 times. Cells were initially seeded in triplicate at 8.0 x 10^3cells/ml in T-25 flasks and counted daily beginning on day 3 for five days. Flasks were incubated at 37ºC in a 5% CO_2 atmosphere. Error bars indicate +/- one standard deviation.

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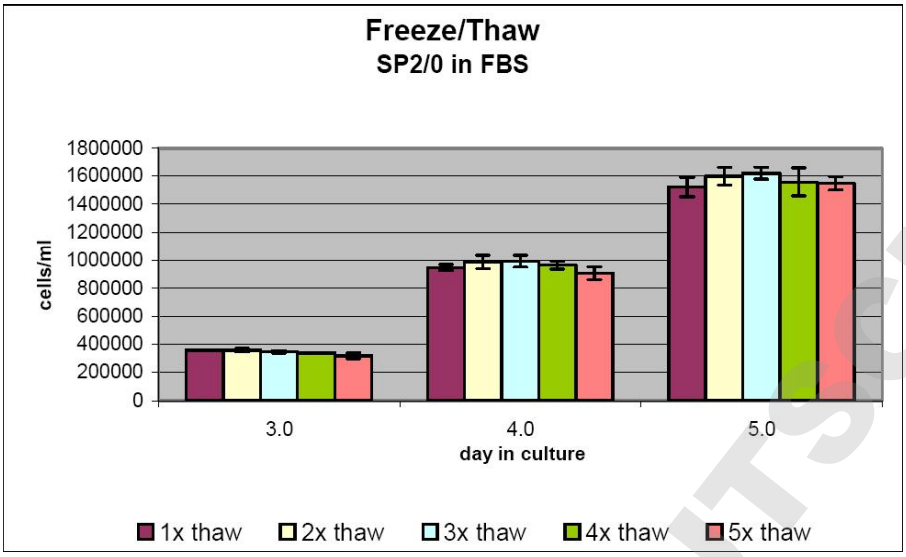

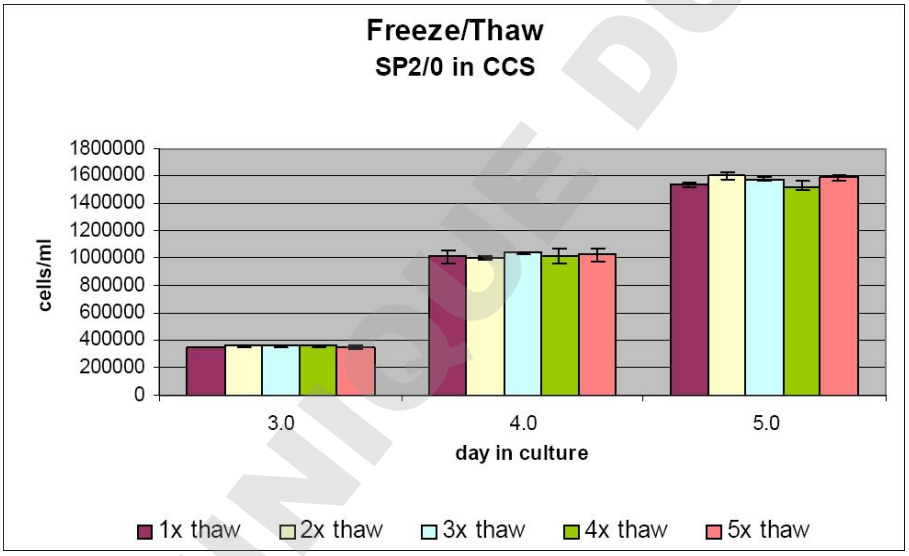

Figure 5: Growth of Sp2/0-Ag14 cells in DME high glucose medium with 10% serum thawed up to 5 times. Cells were initially seeded in triplicate at 8.0 x 10^3cells/ml in T-25 flasks and counted daily beginning on day 3 for five days. Flasks were incubated at 37ºC in a 5% CO_2 atmosphere. Error bars indicate +/- one standard deviation.