QUALITY CONTROL

Cryopreservation Replaces Cell Starvation in Cytokine Proliferation Assays

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INTRODUCTION Proliferation assays require the cells to be in a quiescent, non-dividing state to allow a strong response to the tested mitogen. Therefore, cells from a growing culture need to be arrested in proliferation before use. This is achieved by reduced serum levels or the withdrawal of an essential growth factor.

TF-1 human erythroblasts and NFS-60 murine B-cells depend on growth factors and provide a valid model to determine the potency of respective cytokines. The potency of Sargramostim (GM-CSF), is tested on TF-1, while NFS-60 are used to measure the potency of Filgrastim (G-CSF). Both assays require the starvation of the cells in deprived medium before use.

The use of assay ready cells in bioassays is a well-established approach to improve assay precision and flexibility. The frozen cells are applied instantly after thawing without prior cultivation. Cells get into a quiescent state by cryopreservation starvation, so we here demonstrate that starvation can be dismissed when frozen cells are used instantly after thawing.

Cultured Cells Need to be Starved for Proliferation Assays

NFS-60 cells are cultivated in the presence of murine IL-3 which needs to be deprived before the cells can be used to measure the potency of recombinant G-CSF. Cells are starved in medium with 1% serum and no IL-3 for 24 hours to bring proliferation to a halt. G-CSF is added to reactivate cell division. The increase in cell number over a period of 48 hours is determined by the addition of Resazurin, a metabolic dye. Upon the stimulus of G-CSF cells proliferate in a dose-dependent manner (red). Cells which had not been starved (blue) proliferate already at no or very low concentrations of the cytokine. No G-CSF dependent increase in proliferation can be detected (Fig. 1 A).

Likewise, TF-1 are grown in the presence of cytokine and have to be starved before being used in a GM-CSF proliferation assay. While starved cells respond to GM-CSF in a dose-dependent way (red) the effect on the proliferation is significantly reduced in cells which had not been starved before (Fig. 1 B).

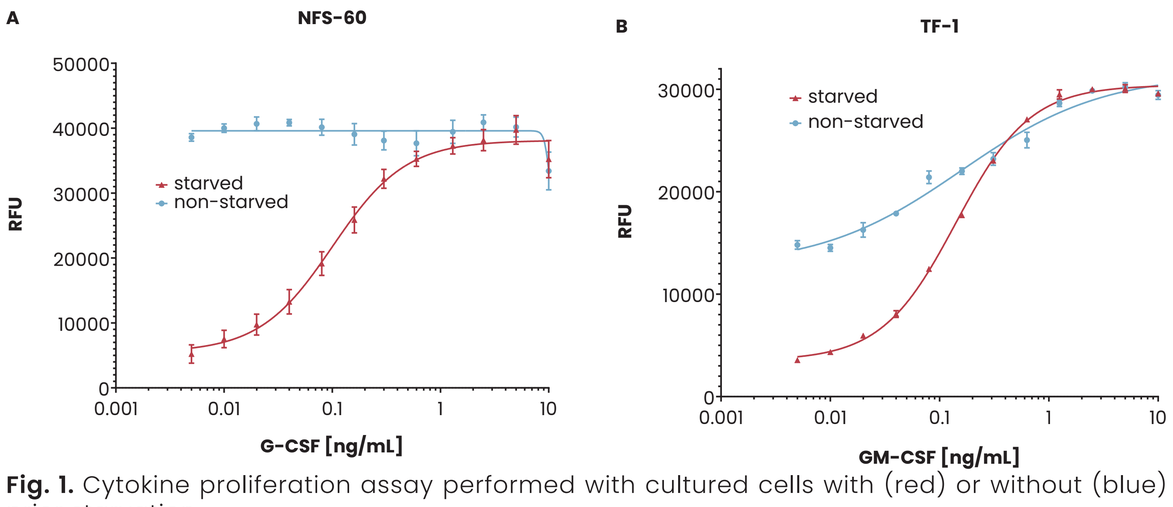

Fig. 1. Cytokine proliferation assay performed with cultured cells with (red) or without (blue) prior starvation.

Cryopreservation of Cells and Assay Ready Use Make a Starvation Dispensable

Cryopreservation arrests cells in proliferation. To test if this makes a starving of the cells unnecessary, assay ready frozen cells had been prepared from NFS-60 and TF-1 cells. After thawing, the cells were seeded into deprived medium and either starved for additional 24 hours or used instantly for the proliferation assay. Increasing doses of G-CSF or GM-CSF were added to the assay ready cells and the assay was performed as described above.

Both, the cells which had been starved as well as the non-starved cells responded to the respective cytokine in the same way (Fig. 2 A + B). NFS-60 and TF-1 assay ready cells did not proliferate in the absence of cytokines and restarted to proliferate when G-CSF or GM-CSF was added, respectively. No reduced response (S/B~ 10) could be observed, suggesting that cryopreservation can replace starvation when the cells are used instantly after thawing.

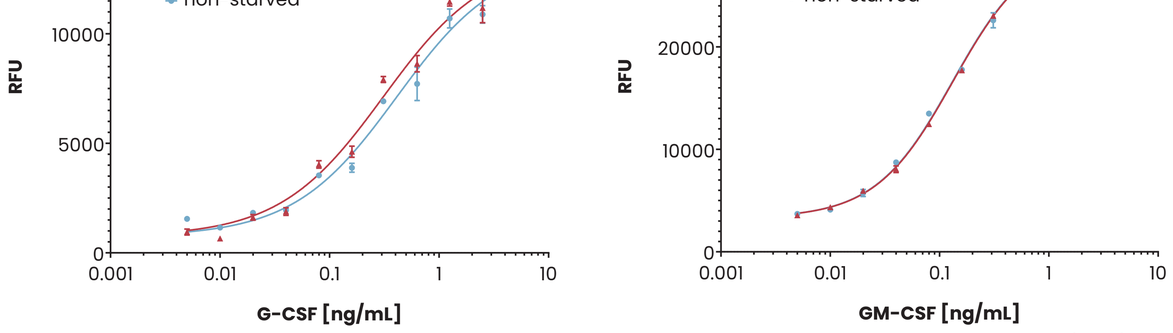

Fig. 2. Cytokine proliferation assay performed with assay ready frozen cells with (red) or without (blue) prior starvation.

Non-Starved Assay Ready Cells Respond to Growth Factors like Cultured Cells after Starvation.

To demonstrate comparability, assay ready NFS-60 and TF-1 were used in the cytokine proliferation assay instantly after thawing without prior cultivation or starving. The results were compared with cells from a continuous culture which had been starved for 24 hours before use (Fig 3 A+B). Frozen assay ready cells respond similar to the cytokines as starved cultured cells. No significant shift in potency can be detected in TF-1. Starved NFS-60 cells from continuous culture display a high background resulting in reduced S/B of 3, while assay ready cells respond 12x over background. A slight shift in EC50 from 0.063ng/ml in starved cultured cells to 0.183ng/ml in assay ready cells was observed.

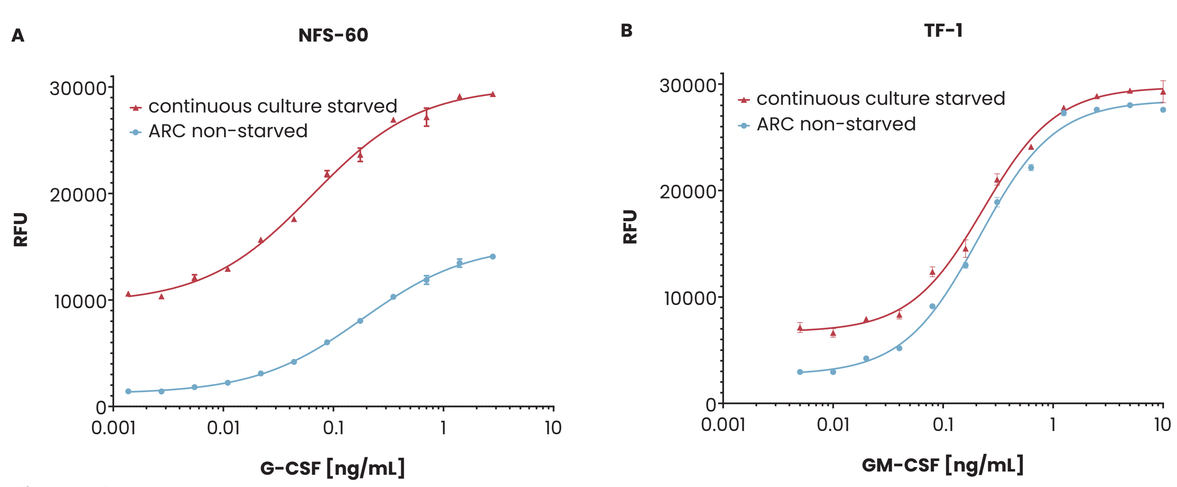

Fig. 3. Comparison of assay performance of starved, continuously cultured cells and non- starved, assay ready cells.

Assay Ready NFS-60 and TF-1 Perform very Reproducibly and Provide Accurate Results

The potency of a given G-CSF or GM-CSF preparation can be precisely determined with NFS-60 or TF-1 assay ready cells. The cells were seeded into assay medium were used instantly in the respective proliferation assay.

Four or five independent experiments on different days revealed a high assay precision, having an average EC50 of 0.228 ng/ml at a CV of 9% in the G-CSF (Fig. 4A) and an average EC50 of 0.038 ng/ml at a CV of 12% in the GM-CSF proliferation assay (Fig 4B). Also the accuracy (data not shown) was very good, ranging from 90% to 107% in the G-CSF recovery and from 87% to 93% recovery in the GM-CSF proliferation assay.

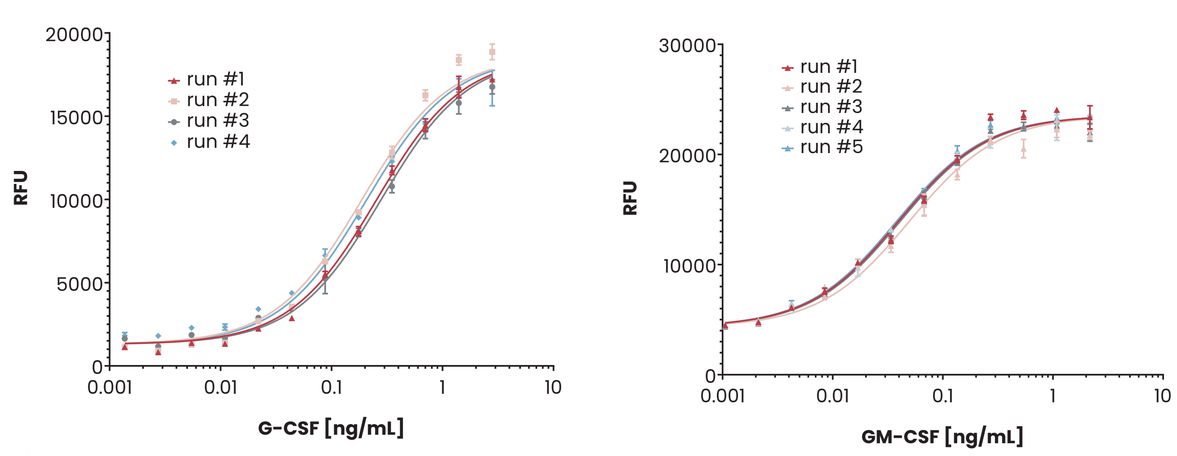

Fig. 4. Precision of assay ready NFS-60 and TF-1 instantly used in cytokine proliferation assays.

CONCLUSION Cryopreserved cells often need time to fully recover after thawing. The cells proceed through a lag-phase until they regain their full logarithmic growth. However, when assay ready cells are used instantly after thawing, we can observe that an optimal freezing and recovery process helps to reduce the lag-phase to obtain an adequate assay window. By this, the arrest in cell division during cryopreservation can be turned into an advantage. When cells from a continuous culture are used in proliferation assay, they need to be starved in deprived assay medium before treated with the mitogenic samples. Here we demonstrated that a freezing of cells can makes this procedure unnecessary. Assay ready cells can be applied instantly in cytokine proliferation assays without starving.

Frozen TF-1 used instantly without starvation respond equally to GM-CFS compared to starved cells from a continuous culture. No potency shift could be detected. Assay ready NFS-60 display a lower background compared to cells from continuous culture despite starvation. Although a shift in potency was detected, the NFS-60 assay ready cells respond with a four times broader assay window. Additionally, assay ready cells provide highly accurate and precise result because variations in cell cultivation, handling, medium and cell passage are consequently excluded from the actual assay procedure. With the otherwise required starving procedure another potential source of assay variations is eliminated.

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Literature

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