Nucleus Biologics

APPLICATION NOTE

Demonstrating Functional Consistency in Cell Culture Reagents for Sensitive Applications, Part 1

Alyssa Master, PhD
Director of Science and Applications, Nucleus Biologics

Introduction

Lack of reproducibility is a known issue in biomedical research. Studies estimate that every year $28B is spent on research that is not reproducible or translatable [1]. Scientists are starting to realize the massive effect reagent variability can have on their research outcomes [2]. Consistency is becoming even more important with the rise in demand for immunology based therapeutics research. Reliable in vitro growth of immune cells such as T and B cells has become a necessity to further this research. The nature of immune cells makes them very sensitive to minute changes in the reagents used for their culture. Thus, learning how to choose and verify that reagents such as fetal bovine serum (FBS) are suitable for culture of immune cells is critical.

The sensitivity of immune cells to their culture conditions leads many researchers to spend valuable time and resources testing multiple reagent lots. Most researchers must test many lots of FBS to find the one that performs consistently in their assays. This is a grueling and time consuming process. In this study, we examined the consistency across several lots of Nucleus Biologics' Single Origin Australia Source FBS (Catalog#: FBS1824), and its effect on T cell proliferation. T cells are activated by foreign antigens, resulting in proliferation and associated cytokine production to effectively neutralize these antigens. Because it comes from a non-human source, all FBS causes a low basal level of activation in human T cells [3]. Despite this, FBS is still commonly used because it is a ubiquitous and cost-effective reagent. However, if the serum used to grow these cells contains immunogenic components, the T cells will become activated above these normal levels, leading to misleading and inconsistent experimental results. Therefore, it is very important that the FBS used to grow T cells is characterized and consistent. Cell proliferation assays will reflect the immunogenicity of the serum.

For this study we utilized T cells isolated from whole blood of different donors. Primary T cell immune responses are more physiologically relevant than those of immortalized T cell lines [4]. Cell lines are easier to work with and often cheaper but rarely behave the same as primary cells because they have been manipulated to grow well in an in vitro setting. Using primary T cells allows for better modeling but creates an additional complication of donor-to-donor variability. Due to this internal variability, we tested three donors to compare trends in the data.

It is a common practice to heat-inactivate FBS that is used to culture immune cells. Heat inactivation involves heating FBS to 56°C to inactivate complement proteins and, in previous decades, mycoplasma. Advancements in filtration technology have made the latter reasoning unnecessary. However, it is ingrained in many research protocols to heat-inactivate serum. It is now becoming more accepted that this may not be necessary. Research has shown that the bovine placental barrier does not allow the interchange of most complement proteins such as IgG, rendering it largely unnecessary to heat-inactivate serum of truly fetal (pre-partum) origin [5]. We utilized both heat-inactivated and untreated FBS for this lot-to-lot consistency study to share some insight on the need (or lack thereof) to heat-inactivate FBS for T cell applications.

[1] Freedman LP, Cockburn IM, Simcoe TS (2015) The Economics of Reproducibility in Preclinical Research. PLoS Biol 13(6): e1002165. https://doi.org/10.1371/journal.pbio.1002165.

[2] Baker, M, (2016) Reproducibility: Respect Your Cells! Nature 537(7620):433-435. https://doi.org/10.1038/537433a

[3] MacDermott, RP, Bragdon, MJ (1983) Fetal Calf Serum Augmentation During Cell Separation Procedures Accounts for the Majority of Human Autologous Mixed Leukocyte Reactivity. Behring Inst Mitt. 72: 122-8.

[4] Yssel, H, Spits, H (2005) Generation and Maintenance of Cloned Human T Cell Lines. Curr Prot Immun. 65(1) https://doi.org/10.1002/0471142735.im0719s47

[5] Cheever, M, Master, A, Versteegen, R (2017) A Method For Differentiating Fetal Bovine Serum from Newborn Calf Serum. BioProcess J 16. https://doi.org/10.12665/J160A.Cheever

Materials & Methods

Whole blood was obtained from three separate donors and T cell harvest began the same day. For each of the donors, peripheral blood mononuclear cells (PBMCs) were isolated using phase separation. CD4+ T cells were further isolated using a negative selection kit from Stem Cell Technologies.

Four lots of Nucleus Biologics FBS were used for this study. The chosen lots spanned over a year of collections so that any seasonal differences in the FBS were accounted for. Nucleus Biologics Single Origin Australian Source FBS (Catalog# FBS1824-001 for untreated and FBS1824-003 for heat inactivated) was heat-inactivated using a standard protocol. Briefly, the FBS was heated to 56°C for 30 minutes in a water bath before being allowed to cool down for use.

For the proliferation assay, cells from each of three donors were seeded in triplicate in 96 well plates and treated with RPMI 1640 with Glutamax and 10% FBS. Heat-inactivated and untreated controls of four different lots of FBS were used. T cells were activated using Dynabeads Human T-Activator CD3/28 (Thermo Fisher Cat. 11131D). Dynabeads were removed on Day 7. Cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) for determining proliferation, grown for 13 days and collected at various time points between Day 0 and Day 13. For each time point, cells were stained for viability and assessed by flow cytometry. Media was changed on day 4, 7 and 11.

One-way ANOVAs were completed to compare untreated FBS to heat-inactivated FBS as well as comparison between lots. They were also run on all donors. Statistical significance was defined as $p<0.05$.

Results & Discussion

We first investigated if different lots of standard untreated Nucleus Biologics FBS would induce similar levels of proliferation in primary T cells. To most closely replicate physiological conditions, we utilized primary T cells isolated from the PBMCs of three human donors. Proliferation curves were generated over 13 days following a 7 day activation. There were no significant differences between the four lots of FBS tested. The figures show data from one donor though the trend was the same across all donors.

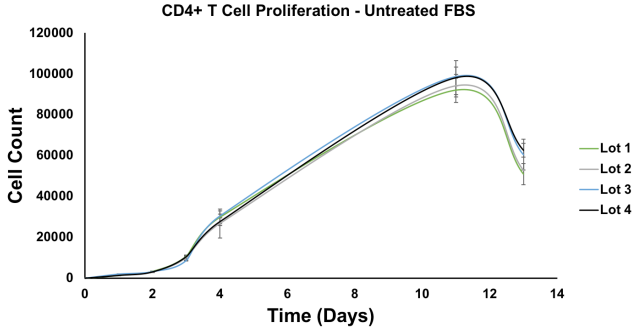

Figure 1. Proliferation of CD4+ T cells grown for 13 days in four different lots of untreated FBS. There was no statistical significance between four untreated lots ($p=0.999$).

Heat inactivation is still commonly used in laboratories worldwide. We next tested if heat-inactivated FBS maintained the same level of consistency as observed in untreated FBS. Indeed, under identical conditions to the first experiment we again saw minimal variability in T cell proliferation across lots (Figure 2).

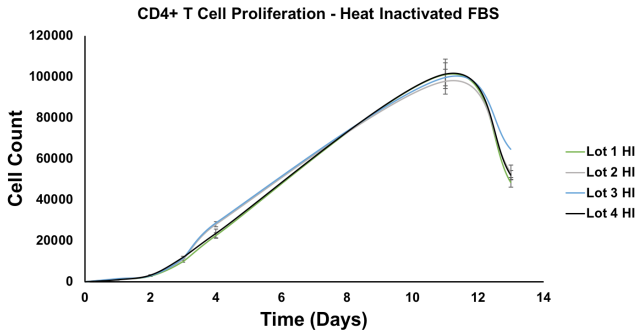

Figure 2. Proliferation of CD4+ T cells grown for 13 days in four different lots of heat-inactivated FBS. There was no statistical significance between four heat inactivated lots (p=0.999).

Finally, we compared untreated and heat-inactivated FBS to determine if heating had altered any proliferation-inducing properties. We observed that there was no difference in performance or consistency between the two conditions.

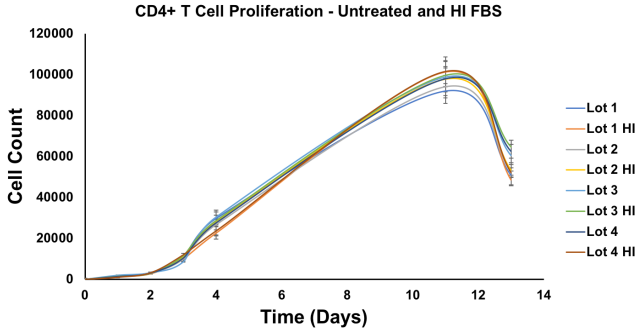

Figure 3. Proliferation of CD4+ T cells grown for 13 days in four different lots of untreated and heat-inactivated FBS. There was no statistical significance between four lots (both heat inactivated and untreated) (p=0.999).

Conclusion

The importance of uniformity in T cell research is paramount [6]. Changing reagents during a study can be disastrous, causing experiments to be unreliable at best and sometimes precluding their completion entirely. Labs often purchase FBS in bulk to avoid switching lots, but even the best-prepared lab will eventually exhaust their supply. The best safeguard against future problems is to use products with minimal variability from lot to lot [2].

In this study, we demonstrate that Nucleus Biologics' Single Origin Australia Source FBS produces highly consistent T cell proliferation across separate production lots as both untreated or heat inactivated. We have shown that if FBS is consistent in manufacturing process and truly of fetal origin, heat-inactivation to prevent immune cell stimulation is largely unnecessary. Importantly, our results were unchanged across multiple human donors as well as lots that were produced over the course of a year. To our knowledge, no FBS manufacturer has been able to show results such as these, particularly in T cells. This is likely because most manufacturers obtain product from multiple sources rather than using single origin sourcing models. This enables researchers working with sensitive primary immune cells to have confidence that their experimental results are accurate, rather than artifacts of a variable product. Observing this result in sensitive primary T cells indicates a high likelihood that similar results will be seen in more robust cell types. The amount of time and energy saved by using proven, consistent reagents, like Nucleus Biologics FBS, is invaluable.

[6] Block, A, Rohde, M, Erben, W, Hammer, M, Hummel, M et al (2008) Impact of Cell Culture Media on the Expansion Efficiency and T-cell Receptor Vbeta Repertoire of In Vitro Expanded T Cells Using Feeder Cells. Med Sci Monit. 14(5): BR88-95.