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General Guidelines for Culture of Multiple Myeloma Cell Lines

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We use this protocol and it's working

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Disclaimer

Abstract

General guidelines for culture and use of myeloma cell lines in the Fruman Lab.

Materials

RPMI 1640 (Hyclone SH30255)
Pen/Strep/Glutamine (Gibco 10378016)
1M HEPES (Corning 25-060-CI)

Troubleshooting

1 **Incubator Settings:** 37 degrees Celsius, 5% CO2

2 **Medium:** 88% RPMI 1640, 10% FBS, 1% Pen/Strep/Glutamine, 1% (Final: 10 mM) of 1M HEPES

3 **Cryopreservation:** Freeze medium: 90% FBS, 10% DMSO. 5 million cells are resuspended in 500uL of freeze media, transferred to cryotubes, and frozen slowly in Mr. Frosty™ Freezing Container at -80 degrees Celsius.

4 **Guidelines for Experimental Use:**

1. Passages are not intuitive for suspension cells and passage number should instead be used to indicate number of times the original stock has been grown out and frozen for future experiments.
2. New cell lines should be grown to at least 35 million cells and a minimum of 6 vials should be frozen for experimental use throughout the year and keeping at least one for long term storage in liquid nitrogen.
3. Cell lines should be authenticated through STR profiling annually or whenever a new set of stocks are cryopreserved for future experiments to confirm lack of cross contamination.
4. Cell lines should not be cultured for longer than 2 months when used for experiments.
5. Cell lines should be maintained at concentrations between 0.5 million to 2 million per mL throughout culture as to not exhaust media.
6. Experiments requiring incubation times less than 24 hours should have cells plated at 1 million per mL media.
7. Experiments requiring incubation times greater than 24 hours should have cells plated at 0.25-0.5 million per mL media.
8. When ordering new lots of FBS, a sample should be requested to test if the new lot will be deleterious to cell survival and cell proliferation.