Mid-term culture of human NKs.

* from PBMCs, lysate red blood cells and resuspend cells at 0.8x106 cells/ml in RPMI 10% FCS
* count irradiated (3000 rads) RPMI 8866 (feeder cells) and resuspend them at 0.2x106 cells/ml in RPMI 10% FCS
* plate in 48 well plates 0.5 ml of PBMCs + 0.5 ml of RPMI 8866
* incubate for 10-12 days (every 3-4 days carefully take out 500 ul of media and re-plate 500 ul fresh media in every well). After 10-12 days NK cells should be around 90% of viable cells. To run cytotoxicity assay pre-activate NKs with 200 U/ml of rh-IL-2 overnight.

Refs:

<http://www.jimmunol.org/cgi/pmidlookup?view=long&pmid=10358164>

[http://onlinelibrary.wiley.com/store/10.1002/1521-4141(200002)30:2%3C644::AID-IMMU644%3E3.0.CO;2-H/asset/644\_ftp.pdf?v=1&t=hovogyc2&s=4bd618ab0974d4019dd7b32c1167535fa53cf782&systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+GMT+%2805%3A00-10%3A00+EST%29+for+essential+maintenance](http://onlinelibrary.wiley.com/store/10.1002/1521-4141%28200002%2930%3A2%3C644%3A%3AAID-IMMU644%3E3.0.CO%3B2-H/asset/644_ftp.pdf?v=1&t=hovogyc2&s=4bd618ab0974d4019dd7b32c1167535fa53cf782&systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+GMT+%2805%3A00-10%3A00+EST%29+for+essential+maintenance)

<http://bloodjournal.hematologylibrary.org/content/110/2/606.long>