Please cite this article as: Li-Ting and Shih-Hsien, (2013). Xenograft Tumor Growth Assay, *Bio-protocol* 3 (20): e948. DOI: 10.21769/BioProtoc.948.

Xenograft Tumor Growth Assay

Li-Ting Wang and Shih-Hsien Hsu*

Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
*For correspondence: jackhsu@kmu.edu.tw

[Abstract] Chronic inflammation drives initiation of hepatocellular carcinoma (HCC), but the underlying mechanisms linking inflammation and tumor formation remain obscure. In this study, Xenograft tumor assay was used to determine the tumorigenic activity of hepatoma cells with ISX over expression on nude mice *in vivo*.

Materials and Reagents

1. HCC cells (Hep G2: ATCC® HB-8065™ and Hep 3B: ATCC® HB-8064™)
2. CAnN.Cg-Foxn1nu/CrlBltw Nude mice
3. ISX fused with GFP or ISX shRNAi expression constructs
4. PBS (MDBio)
5. 2.5% Trypsin (10x) (Gibco , catalog number: 15090-046)
6. MEM Culture medium (Gibco, catalog number: 61100-061)

Equipment

1. 100 mm^2 plate
2. 15 ml conical tubes
3. Centrifuge (Beckman Coulter)
4. Eppendorf
5. 1 ml syringes (0.45 x 13 mm)
6. Tissue culture hood
7. Caliper (Digital Caliper)

Procedure

1. HCC cells were transfected with ISX fused with GFP or ISX shRNAi expression constructs and selected into stable clones.
2. Determining the cells number for injection. 5 x 10^7 cells per ml will be required to trypsinizing (usually a 100% confluent plate of 100 mm^2 will yield at least 6 injections at 5 x 10^6 cells/injection).
3. Trypsinizing the cells with 1x trypsin solution from needed number of plates to be counted all at once.

4. Collecting the detached cells in 15 ml conical tubes and spin for 3 min at 300 *x g*.
5. To remove the supernatant and re-suspend the cells with 3 ml of culture medium for counting.
6. To remove three 10 µl aliquots into 3 separate eppendorfs and dilute each 10 µl 1:100 by adding 990 µl of culture medium, mix well for counting.
7. Then, to remove 10 µl of 1:10 dilutions for counting, counting each of three dilutions and average the three numbers.
8. Determining the concentration of cells in cells/ml by using the following formula: Average counts x 10,000 x dilution factor (1,000) = #cells/ml.
9. Determining the volume required to add to achieve final concentration of cells for injection per volume to be injected (*i.e.* 5 x 10^6 cells/ml injections).
10. Spin down 15 ml conical for 3 min at 300 *x g*.
11. Discard supernatant and re-suspend the pellet in the previously determined volume from step 8.
12. Draw up each injection/mouse in 1 ml syringes in the tissue culture hood prior to going to the animal facility. Place the separate syringes each containing 200 µl on ice.
13. The tumor volume was estimated according to the formula: volume (cm^3) = 1/2(L x W^2), where L and W are the length and width of the tumor, respectively.

Acknowledgments

This protocol was adapted from Hsu *et al.* (2013).

References

1. Hsu, S. H., Wang, L. T., Lee, K. T., Chen, Y. L., Liu, K. Y., Suen, J. L., Chai, C. Y. and Wang, S. N. (2013). Proinflammatory homeobox gene, ISX, regulates tumor growth and survival in hepatocellular carcinoma. *Cancer Res* 73(2): 508-518.

