ELISpot

The ELISpot assay prevails as one of the most sensitive and meaningful assays for the detection of antigen-specific, effector immune responses. Acquisition of cellular analyte for ELISpot analysis is typically not problematic when derived from tissues enriched in lymphocytes (e.g., lymphoid organs and blood); however, cell processing becomes more difficult when lymphocytes represent only a very minor population relative to the source tissue, especially when the source tissue is in limited supply (e.g., small mouse tumors). Traditional enzymatic-based methods for dissociating tumors often result in poor yields, inconsistent lymphocyte enrichment, and can have deleterious effects on lymphocyte phenotype and function. To address these limitations, we have developed an enzyme-free protocol for processing tumor infiltrating lymphocytes (TILs) from small mouse tumors, which enables the enumeration of antigen-specific effector lymphocytes using ELISpot analysis. This procedure is predicated on the dissociation of tumor tissue using gentle agitation with a paddle blender followed by a brief in vitro culture period to remove adherent cells, as well as to revive lymphocytes from a non-responsive state. Although this method is demonstrated with mouse intracerebral tumors, we have found that this protocol is applicable to peripheral tumors and may likely extend to alternative tissue sources wherein lymphocytes exist in low numbers [1)].

1)
Swartz AM, Reap E, Norberg P, Schmittling R, Janetzki S, Sanchez-Perez L, Sampson JH. A simple and enzyme-free method for processing infiltrating lymphocytes from small mouse tumors for ELISpot analysis. J Immunol Methods. 2018 May 30. pii: S0022-1759(18)30070-X. doi: 10.1016/j.jim.2018.05.015. [Epub ahead of print] PubMed PMID: 29859231.