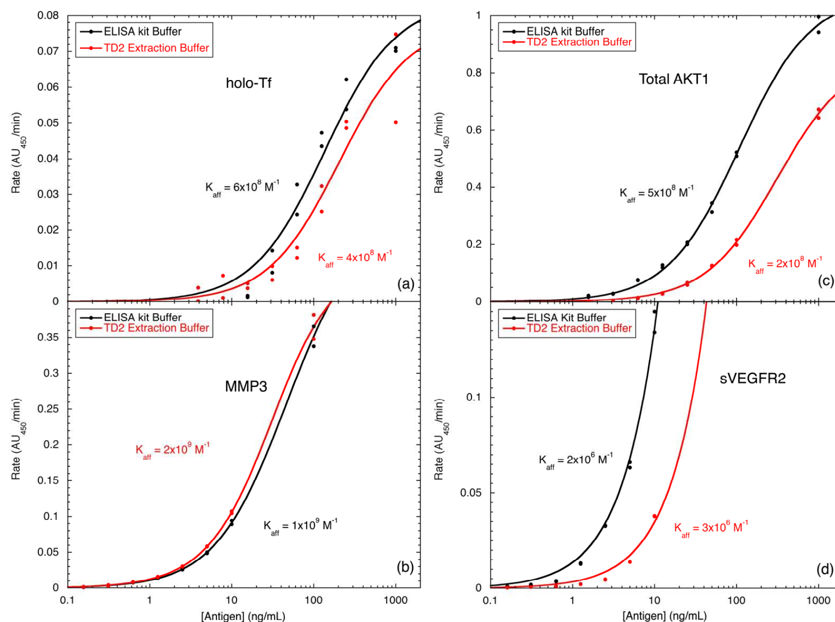
ProteoSolve-TD2 PrEp: Enzyme-Linked Immunosorbant Assays (ELISA) Conducted on Proteins Recovered from Ovarian Tumors Using ProteoSolve-TD2 and Pressure Cycling Technology (PCT)

Introduction

Integral membrane proteins play key biological roles in cell signaling, [1-5] membrane transport, [6-7] as well as pathogen invasion [8-10]. However, clinical exploration of integral membrane proteins has been limited by our ability to recover these proteins in a form suitable for immunoaffinity quantification by ELISA. As such, quantitative clinical assays for this critical class of proteins (e.g., immunosorbant assays) remain elusive, and are generally limited to monitoring serum-soluble extracellular fragments [11-12], or indirect measurement of their mRNAs [13-14].

Here we describe the use of pressure cycling technology (PCT) in combination with PBI's ProteoSolve-TD2 Kit for recovery of both cytosolic and integral membrane proteins from solid ovarian tumors. The ProteoSolve-TD2 buffer system is compatible with downstream immunosorbant assays. This solution allows direct adaptation of commercial ELISA kits developed for measuring serum-soluble membrane protein fragments for the measurement of their integral membrane protein counterparts.



ELISA Results

Figure 1 shows that the ProteoSolve-TD2 buffer system (diluted 1:10 into TDiluent) has negligible effect on the performance of a wide range of ELISAs. Several commercial sandwich ELISA kits—(a) transferrin [Tf], (b) matrix metalloprotease 3 [MMP3], (c) RAC serine/threonine-protein kinases [AKT1], and (d) soluble vascular endothelial growth factor receptor 2 [sVEGFR2]—were used to quantify the effect of the TD2 extraction buffer on subsequent immunoaffinity work. In each case one vial of antigen standards was reconstituted as prescribed in the manufacturer's instructions and a second vial was reconstituted using ProteoSolve-TD2 buffer diluted 1:10 by volume in TDiluent (pH 7.5). The small differences in affinity constants between these buffers appear to lie within the experimental error of the serial dilutions and the curve fitting. Furthermore, there was no consistent trend with K_{aff} being either slightly higher or lower, depending on the assay, and generally within the expected preparation variation of the standards. ProteoSolve-TD2 buffer was used as supplied (not optimized) for each assay.

Western Blots (not shown) indicated that each of the target proteins was recovered nearly quantitatively from the tumor samples. Therefore, it was possible to determine the tissue titers of each of these proteins directly by ELISA (see Table).

Biomarker	Conc. In Extract (ng/mL)	Conc. In Tumor (ng/g)
Holo-Tf	80± 40 x 10^3	5 ±3 x 10^6
MMP3	≈1	≈7
AKT1	40	20 x 10^3
VEGF R2 / sVEGF R2	0.2	1 ± 0.1

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Transferrin (Tf) is a convenient surrogate marker for the blood/serum content of the fresh frozen tumor sample, which was determined to be 15% using the reported serum transferrin concentration value [15]. Most importantly, the assay for soluble vascular endotheilial growth factor receptor 2 (sVEGFR2) was used directly to quantify the tissue titer of the VEGF receptor 2, an integral membrane protein, to be 1±0.1 ng/g of tumor tissue. This value is twice as high as the highest values expected for sVEGFR2 in the 15% blood contamination of the tissue sample [16].

PCT Sample Preparation

Cryogenically-ground, fresh-frozen, tumor samples (200mg) were processed as described in the ProteoSolve-TD2 User's Manual in 1.3 mL of ProteoSolve-TD2 buffer, including treatment with micrococcal nuclease, clarification by centrifugation and where serially-diluted into TDiluent to get into the working range for each assay. A minimum dilution of 1:10 was used as the most concentrated sample. The diluted samples were applied directly to each of the commercial ELISA kits: holo-Tf (Bethyl Labs), MMP3 and sVEGFR2 (R&D Systems), and total AKT1 (Cell Signaling). All kits used a secondary antibody conjugated to horse radish peroxidase. The ELISAs were conducted according to the corresponding kit instructions.

Discussion

ProteoSolve-TD2 and PCT provide a simple and easy to use method for the extraction of proteins, including integral membrane proteins, prior to immunoaffinity techniques. When diluted 1:10 by volume in TDiluent, ProteoSolve-TD2 extracts may be used directly in ELISA assays with little or no change in their sensitivity (either affinity or avidity). Because membrane proteins are extracted together with normal cytosolic proteins and remain soluble in the ProteoSolve buffers, immunoassays designed for serum-soluble membrane proteins can be readily adapted to measure the titers of their integral membrane protein counterparts.

References

1. Rosenbaum et al. Nature (2009) 459 (7245) pp. 356-63.
2. Böhme & Beck-Sickinger. Cell Commun Signal (2009) vol. 7 pp. 16.
3. Flores et al. Curr. Stem Cell Res. Ther. (2009) [Epub].
4. Cotton and Claing. Cell Signal (2009) vol. 21 (7) pp. 1045-53.
5. Vilardaga et al. Mol Endocrinol (2009) vol. 23 (5) pp. 590-9.
6. Gottesman, *Cancer Res., 53:*747-754 (1993).
7. Eck et al., in: Proc. Am. Assoc. Cancer Res., (1993) p. 1388.
8. Abraham and Colonno, J. Virol., (1984) vol. 51, pp. 340-345.
9. Rossmann, Protein Sci., (1994) vol3 pp.1712-1725.
10. Burness, in Virus Receptors, part 2, pp. 64-84, Lonberg-Holm and Phillpson, eds., (Chapman and Hall, 1981).
11. Wojeciech, et al., Oncol. Res. (2007)., vol.16 pp. 445-451.
12. Baron, et al., Idrugs (2009) vol. 12 pp. 302-308.
13. Winston et al., Am. J. Clin. Pathol. (2004) vol. 121 pp. S33-49.
14. Thomas et al., Breast J. (2008) vol. 14 pp. 33-8.
15. Anderson, et al., Mol. Cell. Proteomics (2004), vol. 3 pp. 311-26.
16. Polanski and Anderson (2006) Biomarker Insights, vol. 1 pp. 1-48.