



Protein Analysis: ELISA

Group 3-2:

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Introduction

- ELISA = **E**nzyme-**L**inked **I**mmunosorbent **A**ssay
- Detects and quantifies peptides, proteins, antibodies and hormones
- Performed in 96-well plate
- Combines specificity of antibodies with the sensitivity of simple enzyme assays



Figure 1. ELISA Detector

<https://www.news-medical.net/life-sciences>

Working Mechanism: Direct ELISA

1. Target protein is immobilized in well
2. Enzyme-labeled antibody binds to target protein
3. Non-binding antibodies are washed away
4. Substrate is added and reacts with enzyme, resulting in measurable color change

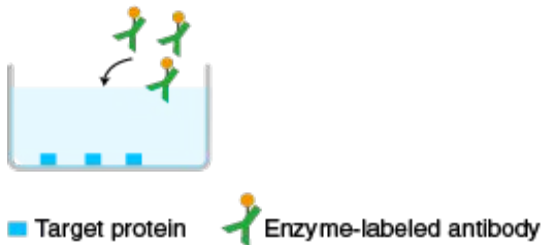


Figure 2. Direct ELISA Mechanism

<https://ruo.mbl.co.jp/bio/e/support/method/elisa>



**Figure 3. Results of ELISA
in 96-Well Plate**

<https://www.researchgate.net>

Four Main Types

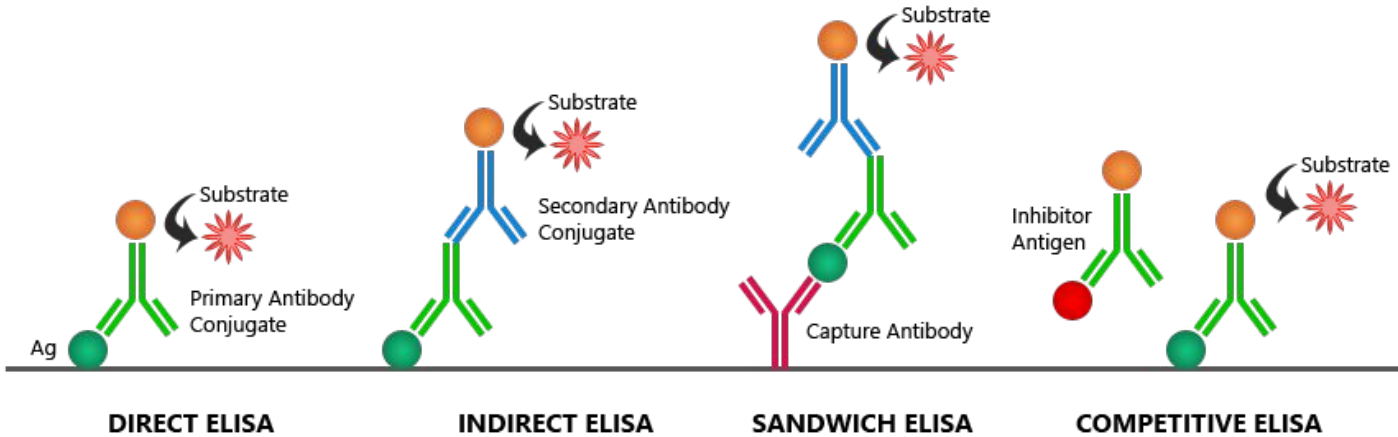


Figure 4. Differences between the Four ELISA Methods

<https://www.researchgate.net>

Mechanism of Sandwich ELISA

1. Coat well with capture antibody and incubate with target protein
2. Non-specific binding sites on antibody are blocked
3. Add enzyme-labeled antibody
4. Detection-specific antibody binds to target protein
5. Enzyme reacts with substrate and produces color change



 Antibody  Target protein

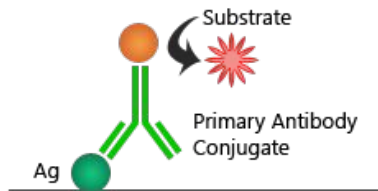
Figure 5. Sandwich ELISA Mechanism

<https://ruo.mbl.co.jp/bio/e/support/method/elisa>

Comparing Direct and Sandwich ELISA

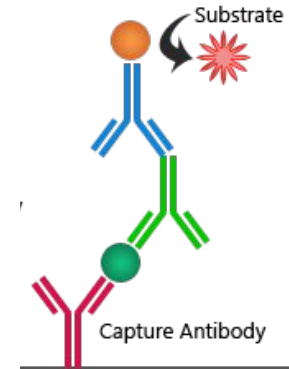
Direct

- Short Protocol
- Less prone to error
- Potential high background noise: antigen immobilization is not specific
- Less flexible



Sandwich

- 2-5X more sensitive
- Suitable for complex samples
- High Specificity: requires 2 antibodies to perform capture and detection
- Flexible: can use both direct and indirect methods





Implemented Technology

Paper Title: Expression of Thymidine Phosphorylase (TP) in Primary Human Renal Cell Carcinoma (RCC) by ELISA Method

- TP expression in 100 samples of RCC tissue and adjacent normal tissue analyzed with ELISA
- No previous treatments for patients with RCC
- Human tissue samples analyzed with ELISA method

Applied Use

Goal: study the expression of TP in patients with RCC (compared to normal tissue)

Methods:

- Homogenize the tissues and centrifuge to remove unwanted components (testing supernatant)
- Sandwich ELISA method:
 - Plate incubated overnight with TP monoclonal antibody (MoAb) 104B
 - Incubated with TP MoAb 232-2 MoAb in a blocking buffer for 2 hours
 - Plate is fully prepped, then incubated with a substrate solution for 5-10 minutes
 - Amount of TP sandwiched between two anti-TP antibodies was measured by absorbance



Figure 6. Scientist Performing ELISA Assay
<https://www.bioagilytix.com/elisa>

Cell Behaviors Studied

- TP is shown to promote angiogenesis, which allows cancer cells to grow and spread to other parts of the body
- ELISA was used to detect the amount of TP enzyme expression in cells
- Studied TP expression in renal cell carcinoma(RCC) and normal adjacent tissue
- Studied the correlation between tumor venous invasion and TP expression

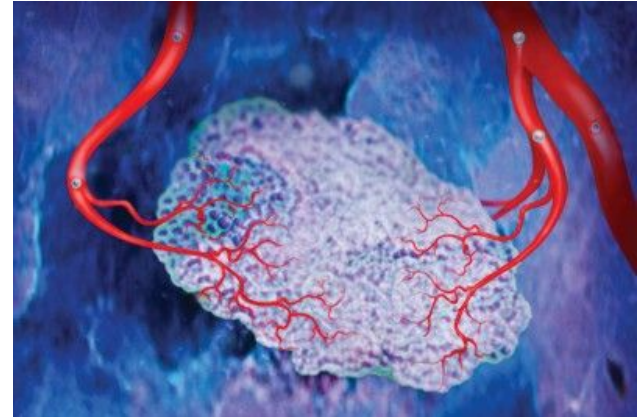


Figure 7. Blood Supply of Tumors
<https://www.mskcc.org/blog/what-angiogenesis>

Analytical Conclusions

- To determine the amount of TP present, its absorbance was measured
- Absorbance was measured against a calibration curve from standard tissue
- Found that TP expression was 9-fold higher in RCC tissue than in normal tissue
- TP expression was higher in patients with tumor venous invasion
- Supports previous findings of TP showing angiogenic activity

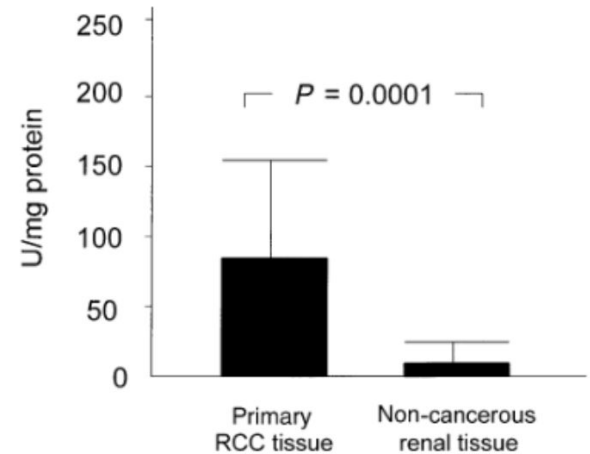


Figure 8. TP Expression in RCC Tissue and Normal Tissue
<https://onlinelibrary.wiley.com>



References

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- [5] Aryal, Sagar. “Sandwich ELISA- Steps and Advantages.” *Microbe Notes*, 12 June 2019, <https://microbenotes.com/sandwich-elisa-steps-and-advantages/>.
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