



Zenyth 340 and Benders p185^{HER-2} Instant ELISA are Innovative Tools for Investigation of Cell Signalling Events Involved in Tumor Biology

1. Introduction

The human HER-2 (c-erbB-2, neu) gene encodes a putative transmembrane growth factor receptor (p185 protein) that is closely related to the epidermal growth factor receptor protein. The HER-2 gene product is a 185 kDa-glycoprotein that contains an extracellular ligand-binding domain and intracellular tyrosine kinase activity (9). p185^{HER-2} protein staining is observed only in low levels in epithelial cells of most organs in normal human tissues and at slightly higher levels in fetal tissues.

Both HER-2 oncogene amplification and oncoprotein overexpression have been analyzed for potential utility in diagnostic and prognostic tests for: breast (5, 6, 8, 11, 14, 18, 19, 21, 23), ovarian (3, 15, 17, 19, 26), gastric (10, 25), lung (7, 12, 22), and other cancers (1, 4, 5, 16, 20, 24). In these malignancies the p185^{HER-2} oncoprotein overexpression is correlated with a poor prognosis.

In 15-40 % of primary breast cancers, amplification of the HER-2 oncogene is found which is highly correlated with overexpression of the encoded 185 kDa protein and seems to play a major role especially during the initiation of ductal carcinomas. p185^{HER-2} overexpression is described as independent prognostic factor with greater predictive power than most of the currently used prognostic tools (21) - especially in axillary lymph-node-positive breast cancer patients.

Studies analyzing small series of patients have suggested a prognostic value for p185^{HER-2} oncoprotein expression in axillary node negative (ANN) patients. An association between oncoprotein expression and decreased overall survival among ANN patients with good nuclear grade tumors has been demonstrated (13). In addition it has been reported that in low risk patients (estrogen receptor positive, small tumors), p185^{HER-2} expression was associated with early recurrence (2). Data demonstrate the large body of evidence implicating p185^{HER-2} oncoprotein in the biology and prognosis of breast carcinoma.

32 % of ovarian carcinomas overexpress the p185^{HER-2} oncoprotein. Survival of those patients is significantly worse compared with cases of normal p185^{HER-2} protein expression. Additionally, patients whose tumors have high p185^{HER-2} protein expression are significantly less likely to have a complete response to primary therapy. Also non-small cell lung cancers which express the p185^{HER-2} protein do

so at higher levels than those found in normal bronchial epithelium, and expression in adenocarcinoma of the lung is independently associated with diminished survival (7). A correlation between p185^{HER-2} expression, and clinical outcome has been also demonstrated for head and neck, salivary gland and placental carcinomas. p185^{HER-2} is useful in identifying cancer cells with increased aggressiveness. Soluble p185^{HER-2} protein levels in serum can be used as diagnostic tool for monitoring the extent of tumor spread, postoperative relapse and/or metastatic risk for different cancers.

2. Materials

- Human p185^{HER-2} ELISA (Bender Medsystems)
- Zenyth 340 Microplate Reader with Evaluation Software
- Adjustable single- and multichannel micropipettes
- Beakers, flasks, cylinders necessary for preparation of reagents

3. Specimen Collection

Cell culture supernatants, human serum, EDTA, or heparinized plasma, amniotic fluid, or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive sp185^{HER-2}. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, frozen sera or plasma should be brought to room temperature slowly and mixed gently.

4. Method

Natural human serum samples were applied to **Bender MedSystems Instant ELISA™** microplates. Alternatively samples from plasma, amniotic fluid or other body liquids can be taken. For incubation times and wash cycles refer to the corresponding instruction manual. Samples were measured with an **Anthos Zenyth 340 reader** at 450nm, reference measurement was taken at 620nm.

5. Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Standard curve is automatically calculated by the Zenyth 340 microplate reader. In addition

the instrument features 4 different modes of curve fitting:

- point to point
 - linear regression
 - cubic spline
 - 4 parameter fit
- To determine the concentration of circulating sp185^{HER-2} for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sp185^{HER-2} concentration.

***Samples have been diluted 1 : 20, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 20).**

- It is suggested that each testing facility establishes a control sample of known sp185^{HER-2} concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

Representative data of standard curve is shown in Figure 1. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Standard	sp185HER- 2 concentration (ng/ml)	O.D. (450nm)	O.D. Mean	C.V. (%)
1	10	2.000	2.026	3.8
	10	2.052		
2	5	1.175	1.200	3.6
	5	1.225		
3	2.5	0.688	0.659	3.8
	2.5	0.629		
4	1.25	0.345	0.349	6.7
	1.25	0.353		
5	0.63	0.207	0.203	2.0
	0.63	0.200		
6	0.32	0.142	0.142	2.1
	0.32	0.142		
7	0.16	0.076	0.084	8.9
	0.16	0.091		
Blank	0.00	0.033	0.040	
	0.00	0.046		

Fig. 1: Shows representative data of a standard curve. Mean values and CV -values were calculated by Zenyth 340 software.

6. Results/Summary:

The ideal platform for absorbance measurement for the **Bender MedSystems Instant ELISA™** turned out to be the **Anthos Zenyth 340 microplate reader**. This absorbance detector allows through its very flexible software a rapid and convenient read out and data processing. **Bender MedSystems' Instant ELISA** technology and the **Anthos reader Zenyth 340** provide a complete solution for busy customers in Biotech and High Throughput laboratories. Both products ensure significant boost of production

7. Literature

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