CD26 is a Key Modulator in Immune Response

1. Introduction

CD26, a T cell activation antigen, is a 110kDa cell surface glycoprotein with known dipeptidyl peptidase IV (DPPIV (12); EC 3.4.14.5) activity in its extracellular domain which is present on various cell types, including T cells and epithelial cells of the liver, kidney, and intestine (9, 13, 17). Of the T cell antigens described to date, CD26 has proved to be one of the most intriguing in the scope of its functional associations. Considerable evidence suggests that CD26 can deliver a potent costimulatory signal to T-cells (8). This signal transducing property appears to be a property of its extracellular domain (5). In addition, CD26 appears to be a functional collagen receptor (3) that may aid activated T-cells in localizing to inflammatory regions (11). It has also been demonstrated that CD26 not only acts as a functional dipeptidyl peptidase IV, but also binds strongly to adenosine deaminase (4).

Significant levels of DPPIV activity have been shown to exist in plasma, serum, and urine (1, 10, 16). The serum form of DPPIV is unique, and is not a breakdown product of membrane CD26, nevertheless exposing significant structural similarity to the membrane form (5, 6).

Like many other T-cell molecules, CD26 is associated with HIV disease progression. There is a correlation of CD26 expression and HIV entry, replication and cytopathicity (15). CD26 has been identified as a key marker for monocytotropic HIV-1 infection, with a mechanism of early loss of CD26 - expressing cells in HIV-1 infected individuals described.
CD26 as an indicator of T-cell activation has been shown to fluctuate in parallel with several autoimmune diseases such as rheumatoid arthritis (14) and autoimmune thyroiditis (7). CD26 has been described as a marker that correlates well with the level of activity of these diseases. It has furthermore been studied as an indicator of the clinical progression in chronic progressive multiple sclerosis (2).

2. Materials

- Human sCD26 ELISA (Bender Medsystems)

- Anthos 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, heparin and citrate plasma or other biological samples will be suitable for use in the assay. Remove serum 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.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive sCD26. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

4. Test Protocol

Natural human serum samples were applied to **Bender MedSystems Instant ELISA^{TM}** microplates. For incubation times and wash cycles refer to the corresponding instruction manual. Samples were measured with a **Zenyth 340** reader at 450nm, reference measurement was taken at 620nm. Alternatively extracts from cell lysates can be applied to microplates.

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 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 sCD26 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 sCD26 concentration.

- Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD26 levels. Such samples require further dilution with sample diluent in order to precisely quantitate the actual sCD26 level.

-It is suggested that each testing facility establishes a control sample of known sCD26 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

Standard	sCD36 concentration (ng/ml)	O.D. (450nm)	O.D. Mean	C.V. (%)
1	500	2.198	2.197	0.1
	500	2.194		
2	250	1.259	1.262	0.3
	250	1.264		
3	125	0.567	0.565	0.6
	125	0.562		
4	62.5	0.246	0.248	0.9
	62.5	0.249		
5	31.3	0.105	0.111	7.0
	31.3	0.116		
6	15.6	0.053	0.058	11.1
	15.6	0.062		
Blank	0	0.022	0.02	
	0	0.017		

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

Results/Summary

The ideal platform for absorbance measurement for the **Bender MedSystems human sCD26 ELISA** turned out to be the **Anthos Zenyth 340 microplate reader.** This absorbance detector allows through 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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