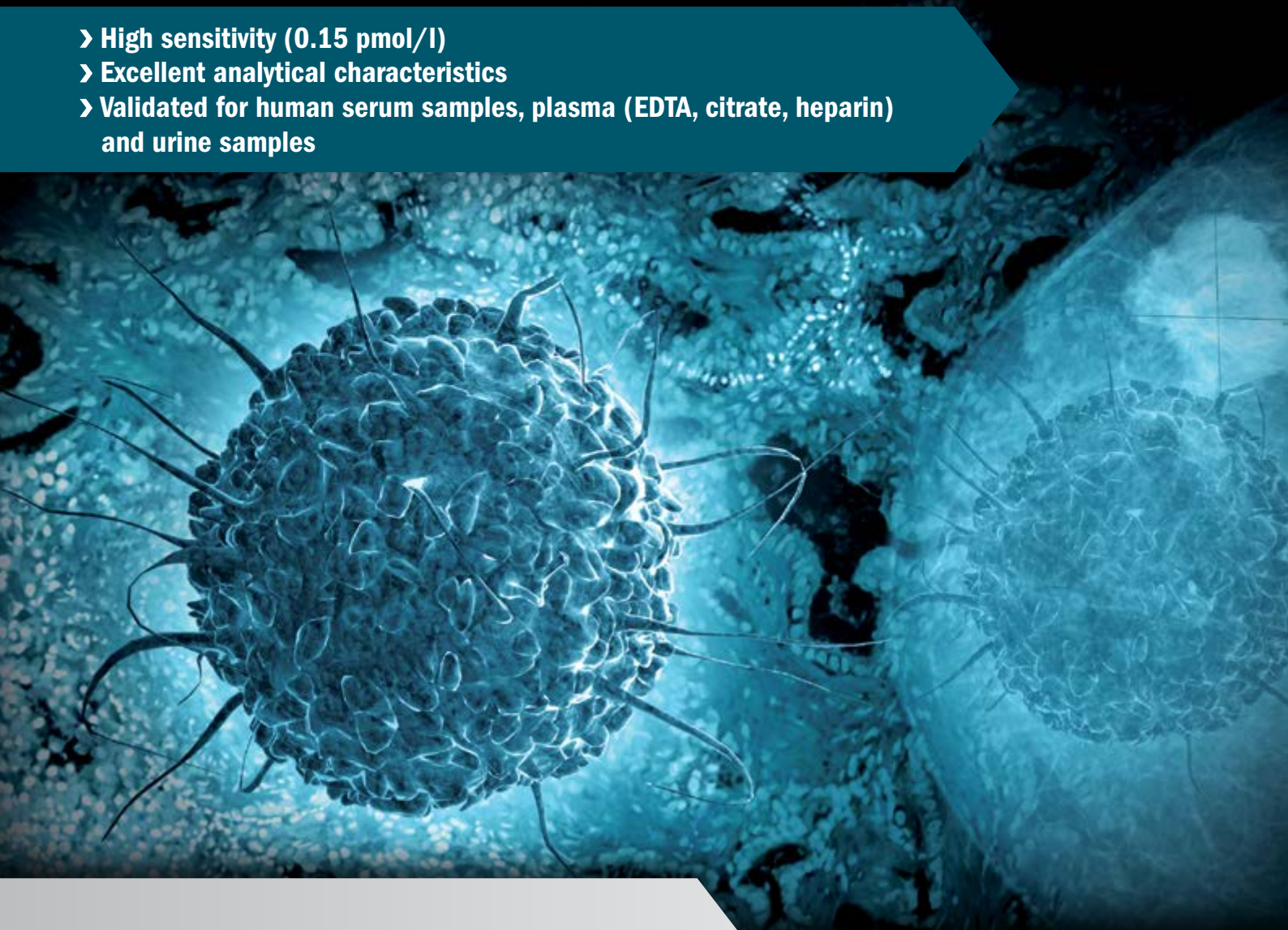


QUANTITATIVE DETERMINATION OF HUMAN EPIDIDYMIS PROTEIN 4

NEW PRODUCT

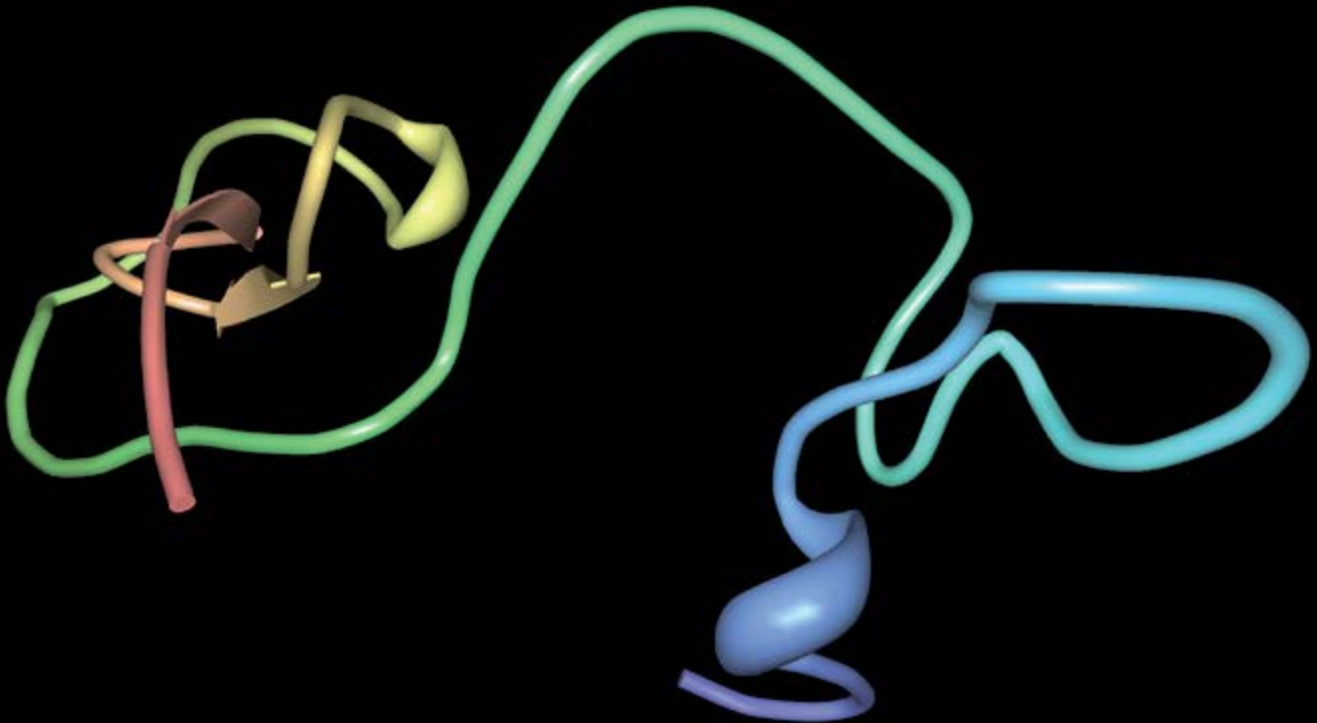
Human Epididymis Protein 4 (HE4) ELISA

- › High sensitivity (0.15 pmol/l)
- › Excellent analytical characteristics
- › Validated for human serum samples, plasma (EDTA, citrate, heparin) and urine samples



ONCOLOGY

HUMAN EPIDIDYMIS PROTEIN 4 (HE4) ELISA



Introduction

Human epididymis protein 4 (HE4) also called whey-acidic-protein (WAP) four-disulfide core domain protein 2 (WFDC2) is a secreted 25 kDa glycoprotein, is a member of the WAP family which was initially described to have tissue specific expression in the epididymis [1]. The gene encoding HE4, WFDC2, is composed by six exons. Five HE4 isoforms have been identified and recognized to define specific patterns differently expressed in neoplasm and normal tissues [2]. Under physiologic conditions, HE4 is reported as protease inhibitor playing a crucial role in sperm maturation [3].

Expression of HE4 has been identified in numerous types of normal human tissues, such as in the epithelium of the respiratory and genitourinary tracts of men and women. An increased HE4 expression has been demonstrated in a range of malignant neoplasms, particularly those of gynecological, pulmonary and gastrointestinal origin [4].

In the last decade, human epididymis protein 4 (HE4) has gained widespread use as an effective tumor biomarker in the diagnosis and follow-up of patients with ovarian cancer [5]. Several clinical studies showed a considerable elevation

of HE4 values in gynecological oncology patients resulting in the firm conclusion that this tumor marker is more specific for ovarian cancer than the commonly used marker CA125 [6, 7].

Recently, human epididymis protein 4 (HE4) has been introduced as a possible novel tumor marker for breast cancer. The preliminary data showed significant elevation of serum HE4 levels in patients with breast cancer compared with those in healthy controls [8].

QUANTITATIVE DETERMINATION OF HUMAN EPIDIDYMIS PROTEIN 4

BioVendor Human Epididymis Protein 4 (HE4) ELISA (RD191369200R)

Intended use

The RD191369200R Human Epididymis Protein 4 (HE4) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human HE4.

- › The total assay time is less than 3.5 hours
- › The kit measures HE4 protein in human serum, plasma (EDTA, citrate, heparin) and urine samples
- › Assay format is 96 wells
- › Standard is recombinant protein based
- › Components of the kit are provided ready to use, concentrated or lyophilized

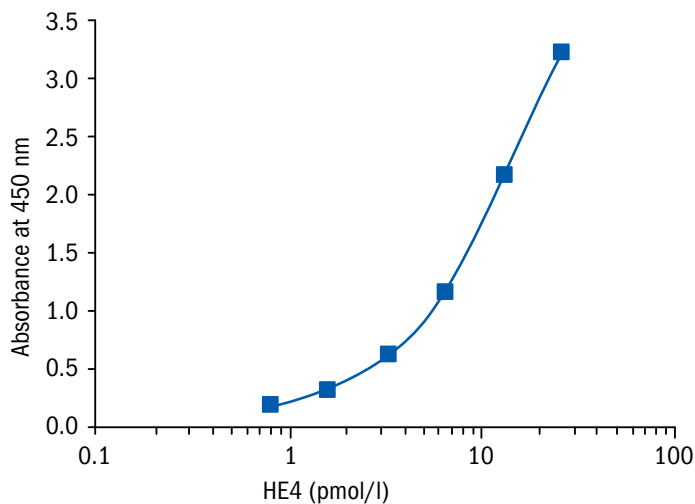
Clinical application

- › Oncology

HUMAN EPIDIDYMIS PROTEIN 4 (HE4) ELISA CAT. NO.: RD191369200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Serum, plasma, urine
Standards	0.82 to 26 pmol/l
Limit of detection	0.15 pmol/l

Test principle

In the BioVendor Human Epididymis Protein 4 (HE4) ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-HE4 antibody. After 60 minutes incubation and washing, biotin labelled rabbit polyclonal anti-HE4 antibody is added and incubated for 60 minutes with captured HE4. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of HE4. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.



HUMAN EPIDIDYMIS PROTEIN 4 (HE4) ELISA

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (pmol/l)	SD (pmol/l)	CV (%)
1	23.9	0.9	3.9
2	42.8	2.4	5.7

Inter-assay (Run-to-Run) (n=6)

Sample	Mean (pmol/l)	SD (pmol/l)	CV (%)
1	35.3	1.5	4.2
2	47.8	2.7	5.7

Spiking recovery

Samples were spiked with different amounts of human HE4 and assayed.

Sample	Observed (pmol/l)	Expected (pmol/l)	Recovery O/E (%)
Serum 1	19.0	-	-
	38.9	35.0	111.0
	55.8	51.0	109.4
	69.4	83.0	83.6
Serum 2	49.1	-	-
	73.8	81.1	91.0
	95.5	113.1	84.4
	156.7	177.1	88.5

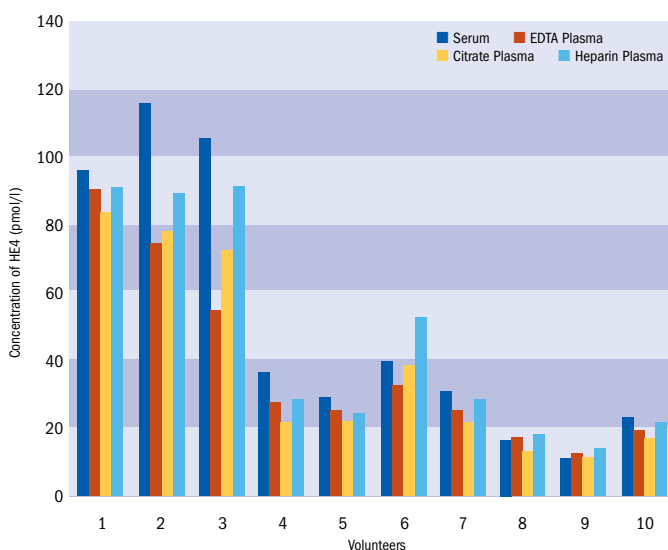
Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pmol/l)	Expected (pmol/l)	Recovery O/E (%)
Serum 1	-	56.7	-	-
	2x	26.6	28.3	93.9
	4x	14.8	14.2	104.7
	8x	7.3	7.1	103.3
Serum 2	-	78.9	-	-
	2x	39.8	39.5	100.8
	4x	20.1	19.7	101.8
	8x	11.8	9.9	119.6

Effect of sample matrix

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 10 individuals. Results are shown below:



Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples
- Add 100 µl Standards and samples
- Incubate at RT for 1 hour with shaking 300 rpm
- Wash plate 3 times
- Prepare Biotin Labelled Antibody Solution
- Add 100 µl Biotin Labelled Antibody
- Incubate at RT for 1 hour with shaking 300 rpm
- Wash plate 3 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min with shaking 300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results

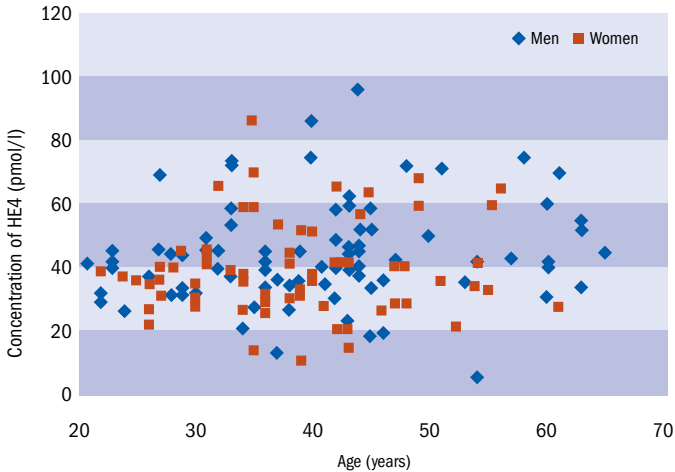
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Preliminary Population Data

The following results were obtained when serum samples from 152 unselected donors (87 men + 65 women) 21-65 years old were assayed with the Biovendor Human Epididymis Protein 4 (HE4) ELISA in our laboratory.

Age and Sex Dependent Distribution of HE4

Sex	Age (years)	n	Mean HE4 (pmol/l)	Median HE4 (pmol/l)	SD HE4 (pmol/l)	Min. HE4 (pmol/l)	Max. HE4 (pmol/l)
Male	21-29	16	39.5	39.6	9.8	26.4	69.4
	30-39	25	41.4	39.4	13.5	13.0	73.8
	40-49	30	47.2	44.1	17.3	18.6	95.0
	50-65	16	46.8	43.7	16.5	6.2	73.6
Female	22-29	13	35.1	35.8	5.7	22.2	44.8
	30-39	24	41.4	38.3	17.2	11.0	86.0
	40-49	20	57.7	40.1	14.7	15.0	383.4
	50-61	8	39.8	35.1	13.9	22.0	64.8



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