



Manual

# Bone Sialoprotein (BSP)

RIA

*For in-vitro-determination of BSP from serum*

(For research use only)

Article No.: K 4221  
Quantity: 100 Tests  
Storage: 2-8 °C

Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim  
Tel.: ++49 6251 70190-0  
Fax: ++ 49 6251 849430  
e.mail: [Immundiagnostik@t-online.de](mailto:Immundiagnostik@t-online.de)  
[www.Immundiagnostik.com](http://www.Immundiagnostik.com)

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## 1. INTENDED USE

The *Immundiagnostik* Assay is intended for the quantitative determination of the BSP in serum. For research use only.

## 2. SUMMARY AND EXPLANATION OF THE TEST

Bone Sialoprotein (BSP) is beside Osteocalcin and Osteonectin the major noncollagenous protein in extracellular matrix of bone. It is a phosphorylated glycoprotein with an approximately molecular weight of 70-80 kDa. The protein has been found in osteoblasts and osteocytes. Diseases concerning the bone turnover, pathological bone alterations as well as the rapid increase of osteoporosis make it necessary to establish new markers. Bone Sialoprotein is discussed as a potential serum marker for monitoring bone remodelling.

## 3. PRINCIPLE OF THE TEST

This **Radio Immuno Assay (RIA)** serves the quantitative determination of Bone Sialoprotein in serum. BSP in patient samples competes with the <sup>125</sup>I labelled BSP tracer for the antibody binding sites. The BSP-tracer-antibody complexes are separated from the unbound fraction using double antibody precipitation. The radioactivity in the precipitated pellet is indirectly proportional to the BSP content .Indications:

#### 4. MATERIAL SUPPLIED

Catalogue No	Kit Components	Quantity
K 4221R	Polypropylen tubes, 75x12 mm	100
K 4221WP	wash buffer, ready-to-use	1 x 110 ml
K 4221TR	<sup>125</sup> I-BSP Tracer	1 x 11 ml
K 4221AK1	primary antibody, ready-to-use	1 x 11 ml
K 4221PA	secondary antibody, ready to use	1 x 50 ml
K 4221ST	Calibrators and NSB, ready to use (1.8; 3.75; 7.5; 15; 30; 60; 120 µg/l)	8 x 500 µl
K 4221ST0	Calibrator 0 , ready for use	1 x 10 ml

#### 5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Gamma Counter
- RIA-tube rack
- Precisions Pipettors and/or multi –channel dispenser

#### 6. PREPARATION AND STORAGE OF REAGENTS

The test reagents are sufficient for 100 tests. They can be stored at 2-8 °C up to the given expiry date (see label of the test package)

#### 7. SPECIMEN COLLECTION AND PREPARATION

BSP-determination can be performed by using **serum only**. The serum is stable for approximately 14 days at 2-8 °C; for longer time the serum should be stored at -20°C. Please clear liphemic samples before determination with this assay.

## 8. ASSAY PROCEDURE

1. Pipette **100 µL** standards, NSB and sample in RIA tubes.
2. Add **100 µL** primary antibody (except NSB; add 100 µl of standard 0 to NSB).
3. Add **100 µL** tracer (<sup>125</sup>I; red colour).
4. Incubate for 24 hours at 4 °C.
5. Add **500 µL** secondary antibody (**mix well before use!**).
6. Incubate 1 hour at 4 °C.
7. Centrifuge 10 min. at 3000 x g; aspirate the supernatant.
8. Add **1 mL** washing buffer to the sediment
9. Centrifuge 10 min. at 3000 x g; aspirate the supernatant.
10. Count the sediment in the Gamma-Counter.

## 9. RESULTS

Determine the mean values of the measured cpm for each standard and sample. The measured mean values or % B/T-values of the standards are drawn on logarithmic paper against the standard concentration to receive a standard curve. The sample concentration are calculated by using the curve.

All samples with a BSP-concentration higher than the highest standard have to be diluted with the standard 0 and tested once more.

## 10. QUALITY CONTROL

### *Sensitivity*

The lowest BSP-value which can be clearly distinguished from standard 0 is 0.7 µg/L .

### *Cross reactivity*

This assay shows no cross reaction with Osteocalcin, Osteonectin or bone specific alkaline phosphatase.

### *Recovery*

To determine the recovery a normal serum is spiked with a known concentration of a BSP standard solution. The table shows the measured, expected and calculated values. The recovery is given in %.

BSP-Spike [µg/L]	measured value [µg/L]	calculated value [µg/L]	Recovery [%]
0,94	5.5	5.5	100.2
1.88	6.8	6.4	106.3
3.75	10.0	8.3	120.4
7.5	14.1	12.1	116.7
15	21.6	19.6	109.4
30	29.6	34.6	85.7
60	51.3	64.6	79.4

*Precision and reproducibility***Intra-Assay-Varianz (Precision):**

Sample	n	Mean value µg/L	Variation- coefficient %
I	12	10.9	7.0
II	12	38.8	6.1

**Inter-Assay-Varianz (Reproducibility):**

Sample	n	Mean value µg/L	Variation- coefficient %
I	9	11.0	9.2
II	9	39.0	9.4

*Normal range*

Normal values range between 5.0 and 21.6 µg/L; the mean values is  $12.1 \pm 5.0$  µg/L. We recommend that each lab should establish its own internal normal range.

## 11. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components which are made of human serum are tested for Australia antigen and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory working should be observed.
- Reagents of the test package contain sodium azide as a bactericide. Contact with skin or mucous membranes has to be avoided.
- All reagents in the test package are to be used for in-vitro diagnostics only.
- The reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the results of the test. Immundiagnostik can therefore not be held reliable for any damage resulting from this.