# Access
Immunoassay Systems

# VITAMIN $B_{12}$

**REF 33000**

## Intended Use

The Access Vitamin $B_{12}$ assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of vitamin $B_{12}$ levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

## Summary and Explanation

Vitamin $B_{12}$ is the name given to any one of a group of substances termed cobalamins. They are composed of a tetrapyrole ring surrounding a central cobalt atom and differ with respect to the side groups attached to the cobalt atom. The predominant form in serum is methylcobalamin while the predominant cellular form is 5′ deoxyadenosylcobalamin.[1] Cyanocobalamin (MW 1355) is the most stable and is used as a reference compound for measuring serum cobalamin concentrations.

Cobalamins are obtained from animal products such as meat, eggs, milk, and other dairy products. When ingested, they are bound by a protein termed intrinsic factor in the gastric juice of the stomach and are subsequently absorbed in the ileum. Intrinsic factor is required for absorption. Once in circulation, cobalamins are taken up and stored in the liver. They are released into the plasma as needed where they are carried by $B_{12}$ binding proteins (transcobalamins).[1]

Vitamin $B_{12}$ is a coenzyme that is involved in two very important metabolic functions vital to normal cell growth and DNA synthesis: 1) the synthesis of methionine, and 2) the conversion of methylmalonyl CoA to succinyl CoA. Deficiency of this vitamin can lead to megaloblastic anemia and ultimately to severe neurological problems.[2,3] Megaloblastic anemia is characterized by the enlargement and reduction in number of all rapidly proliferating cells of the body, including marrow cells, and is primarily a result of the decreased capacity for DNA synthesis. Because vitamin $B_{12}$ and folic acid are linked by the reaction pathway for methionine synthesis, a deficiency in either will disrupt this metabolic pathway and lead to the same symptoms and medical problems. It is usually necessary to measure both vitamins in a clinical workup, with the treatment depending on which of the two is deficient.

Vitamin $B_{12}$ deficiency can occur for one of several reasons.[1,2,4] The most common cause is a defect in the secretion of intrinsic factor, resulting in inadequate vitamin $B_{12}$ absorption from foods. This condition is called pernicious anemia and is most common in people over age 50.[3] Other causes of vitamin $B_{12}$ deficiency are gastrectomy, malabsorption due to surgical resections, and a variety of bacterial or inflammatory diseases affecting the small intestine.[1] The amount of vitamin $B_{12}$ absorbed is directly proportional to the length of functional intestine. Vitamin $B_{12}$ deficiency due to insufficient dietary intake is rare and can occur only after years of abstinence from all animal products.

Elevated levels of vitamin $B_{12}$ have been associated with pregnancy, the use of oral contraceptives and multivitamins, and in myeloproliferative diseases such as chronic granulocytic leukemia and myelomonocytic leukemia. An elevated vitamin $B_{12}$ level in itself has not been known to cause clinical problems.

## Principles of the Procedure

The Access Vitamin $B_{12}$ assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel along with alkaline potassium cyanide and dithiothreitol. This treatment denatures $B_{12}$ binding proteins and converts all forms of vitamin $B_{12}$ to the cyanocobalamin form. After neutralization, intrinsic factor-alkaline phosphatase conjugate and

paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-intrinsic factor are added to the sample. Vitamin $B_{12}$ in the sample binds to the intrinsic factor conjugate, preventing the conjugate from binding to the solid phase anti-intrinsic factor. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of vitamin $B_{12}$ in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

## Product Information

**Access Vitamin $B_{12}$ Reagent Pack**

**Cat. No. 33000: 100 determinations, 2 packs, 50 tests/pack**

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 14 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

| | |
|---|---|
| **R1a:** | Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-intrinsic factor complexes, TRIS buffered saline, surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin** 300. |
| **R1b:** | Borate buffer with surfactant, cobinamide, and < 0.1% sodium azide. |
| **R1c:** | Porcine intrinsic factor-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, surfactant, human serum albumin (HSA), < 0.1% sodium azide, and 0.25% ProClin 300. |
| **R1d:** | 0.5N sodium hydroxide solution (NaOH) with 0.005% potassium cyanide (KCN), CORROSIVE. |
| **R1e:** | 0.02% acetic acid solution with dithiothreitol (DTT). |

## Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[5]
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]

- Xi. Irritant: 0.5N NaOH.

R 36/38: Irritating to eyes and skin.
S 26-37/39: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection.

- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

## Specimen Collection and Preparation

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[7]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 24 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Avoid assaying hemolyzed samples.

## Materials Provided

R1 Access Vitamin $B_{12}$ Reagent Packs

## Materials Required But Not Provided

1. Access Vitamin $B_{12}$ Calibrators
   Provided at zero and approximately 100, 250, 500, 900, and 1500 pg/mL (74, 184, 369, 664, and 1107 pmol/L)
   Cat. No. 33005
2. Quality Control (QC) materials: commercial control material.
3. Access Sample Diluent A
   Vial Cat. No. 81908
   Diluent Pack Cat. No. A79783 (For use with the UniCelDxI system onboard dilution feature.)
4. Access Substrate
   Cat. No. 81906
5. **Access, Access 2, SYNCHRON LXi:**
   Access Wash Buffer II, Cat. No. A16792
   **UniCel DxI:**
   UniCel DxI Wash Buffer II, Cat. No. A16793

6. Access Vitamin $B_{12}$ Calibrator S0
   Cat. No. 33006

**Procedural Comments**

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use forty-five (45) µL of sample for each determination in addition to the sample container and system dead volumes. Use sixty-six (66) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 0.7378.

**Procedure**

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

**Calibration Details**

An active calibration curve is required for all tests. For the Access Vitamin $B_{12}$ assay, calibration is required every 21 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

**Quality Control**

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[8] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

**Results**

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

**Limitations of the Procedure**

1. Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 50–1500 pg/mL [37–1107 pmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 50 pg/mL [< 37 pmol/L]). When the DxI system onboard dilution feature is used, the system will report results as less than 1275 pg/mL (941 pmol/L).

- If a sample contains more than the stated value of the highest Access Vitamin $B_{12}$ Calibrator (S5), report the result as greater than that value (i.e., > 1500 pg/mL [> 1107 pmol/L]). Alternatively, dilute one volume of sample with 4 volumes of Access Vitamin $B_{12}$ Calibrator S0 (zero) which is also available as Access Vitamin $B_{12}$ Calibrator S0, Cat. No. 33006 or dilute one volume of sample with 4 volumes of Access Sample Diluent A, Cat No. 81908. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
  The DxI system onboard dilution feature automates the dilution process, using one volume of sample with 4 volumes of Access Sample Diluent A, allowing samples to be quantitated up to approximately 7500 pg/mL (5533 pmol/L). The system reports the results adjusted for the dilution.

2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[9,10]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. Approximately 50% of patients with pernicious anemia have intrinsic factor antibodies.[11] The initial denaturation step in the Access Vitamin $B_{12}$ assay inactivates intrinsic factor blocking antibodies. However, in very rare cases, some samples may not be inactivated due to the heterogeneity or extremely high titer of the intrinsic factor antibodies.[12] Such interfering antibodies may cause erroneous results. Patients should be further evaluated if suspected of having these antibodies or if the Vitamin $B_{12}$ results are in conflict with other clinical or laboratory findings.
4. The Access Vitamin $B_{12}$ results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.
5. This assay is not validated for testing neonatal or myeloproliferative syndrome specimens for vitamin $B_{12}$ levels.

## Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Sera from 106 normal subjects and 60 diagnosed vitamin $B_{12}$ deficient patients were assayed to establish expected ranges. The diagnosis of vitamin $B_{12}$ deficiency was based on mean corpuscular volume, hematocrit, the presence of megaloblastic cells in bone marrow aspirates, and by vitamin $B_{12}$ RIA. A non-parametric estimate at the 95% confidence level yields the following ranges:

| Units | Normal Range | Indeterminate Range | Deficient Range |
|---|---|---|---|
| pg/mL | 180–914 | 145–180 | ≤ 145 |
| pmol/L | 133–675 | 107–133 | ≤ 107 |

3. Evaluation of vitamin $B_{12}$ deficiency should not depend on results from a single test. Complete evaluation should include other deficiency function tests and results from a physician's clinical evaluation.

## Specific Performance Characteristics

### Methods Comparison

A comparison of 161 values using the Access Vitamin $B_{12}$ assay on the Access Immunoassay System and a commercially available immunoassay kit gave the following statistical data:

| n | Range of Observations (pg/mL) | Intercept (pg/mL) | Slope | Correlation Coefficient (r) |
|---|---|---|---|---|
| 161 | 75–1446 | -20.0 | 0.95 | 0.97 |

A comparison of 46 values obtained by assaying clinical samples of serum and plasma (heparin) using the Access Vitamin $B_{12}$ assay kit gave the following statistical data:

| n | Range of Observations (pg/mL) | Intercept (pg/mL) | Slope | Correlation Coefficient (r) |
|---|---|---|---|---|
| 46 | 141.37–543.30 | 46.4 | 0.86 | 0.937 |

### Dilution Recovery (Linearity)

Volumetric dilution of three samples containing various levels of Access Vitamin $B_{12}$ Calibrator S0 (zero) gave the following data:

| Sample 1 | Expected Concentration (pg/mL) | Determined Concentration (pg/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 1405 | N/A |
| 1/2 | 703 | 744 | 106 |
| 1/3 | 468 | 549 | 117 |
| 1/5 | 281 | 307 | 109 |
| 1/10 | 141 | 137 | 97 |
| 1/15 | 94 | 108 | 115 |
| | | **Mean % Recovery** | **109** |

| Sample 2 | Expected Concentration (pg/mL) | Determined Concentration (pg/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 1451 | N/A |
| 1/2 | 726 | 799 | 110 |
| 1/3 | 484 | 523 | 108 |
| 1/5 | 290 | 294 | 101 |
| 1/10 | 145 | 148 | 102 |
| 1/15 | 97 | 105 | 108 |
| | | **Mean % Recovery** | **106** |

| Sample 3 | Expected Concentration (pg/mL) | Determined Concentration (pg/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 989 | N/A |
| 1/2 | 495 | 476 | 96 |
| 1/3 | 330 | 348 | 105 |
| 1/5 | 198 | 208 | 105 |
| 1/10 | 99 | 119 | 120 |
| 1/15 | 66 | 74 | 112 |
| | | **Mean % Recovery** | **108** |

### Spiking Recovery

Addition of four different levels of cyanocobalamin to two patient samples with low vitamin $B_{12}$ concentrations resulted in the following data:

| Sample 1 (pg/mL) | Expected Concentration (pg/mL) | Determined Concentration (pg/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 91 | N/A |
| 100 | 191 | 194 | 102 |
| 400 | 491 | 520 | 106 |
| 800 | 891 | 966 | 108 |
| 1200 | 1291 | 1323 | 102 |
| | | **Mean % Recovery** | **105** |

| Sample 2 (pg/mL) | Expected Concentration (pg/mL) | Determined Concentration (pg/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 105 | N/A |
| 100 | 205 | 180 | 88 |
| 400 | 505 | 494 | 98 |
| 800 | 905 | 923 | 102 |
| 1200 | 1305 | 1373 | 105 |
| | | **Mean % Recovery** | **98** |

### Imprecision

This assay exhibits total imprecision of < 12% across the assay range. One study, using commercially available human serum based control material, performed by running two replicates of each sample per assay in two assays per day, provided the following data. The data were analyzed via analysis of variance (ANOVA):[13,14]

| Sample | n | Grand Mean (pg/mL) | Within Run (%CV) | Total Imprecision (%CV) |
|---|---|---|---|---|
| 1 | 20 | 88 | 5.0 | 8.5 |
| 2 | 40 | 374 | 4.8 | 6.6 |
| 3 | 40 | 775 | 6.9 | 7.5 |
| 4 | 40 | 975 | 11.4 | 11.4 |

### Analytical Specificity/Interferences

Samples containing up to 10 mg/dL (171 μmol/L) bilirubin, 9 g/dL (90 g/L) total protein, and lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triglycerides do not affect the concentration of vitamin $B_{12}$ assayed.

Serum samples spiked with 10,000 pg/mL (7378 pmol/L) of the vitamin $B_{12}$ analog cobinamide exhibit < 0.5% cross-reactivity.

### Analytical Sensitivity

The lowest detectable level of vitamin $B_{12}$ distinguishable from zero (Access Vitamin $B_{12}$ Calibrator S0) with 95% confidence is 50 pg/mL (37 pmol/L). This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

# Access
Immunoassay Systems

# VITAMIN $B_{12}$ CALIBRATORS

## REF 33005

**Intended Use**

The Access Vitamin $B_{12}$ Calibrators are intended to calibrate the Access Vitamin $B_{12}$ assay for the quantitative determination of vitamin $B_{12}$ levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

**Summary and Explanation**

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

**Traceability**

The measurand (analyte) in the Access Vitamin $B_{12}$ Calibrators is traceable to the manufacturer's working calibrators, which are prepared from purified cyanocobalamin (vitamin $B_{12}$) whose value is determined by spectrophotometry. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

**Product Information**

**Access Vitamin $B_{12}$ Calibrators**
**Cat. No. 33005: S0–S5, 4.0 mL/vial**

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card for exact concentrations.

| | |
|---|---|
| **S0:** | Buffered matrix with human serum albumin (HSA), < 0.1% sodium azide, and 0.25% ProClin** 300. Contains 0.0 pg/mL (pmol/L) vitamin $B_{12}$. |
| **S1, S2, S3, S4, S5:** | Vitamin $B_{12}$ at levels of approximately 100, 250, 500, 900 and 1500 pg/mL (74, 184, 369, 664 and 1107 pmol/L), respectively, in buffered matrix with HSA, < 0.1% sodium azide, and 0.25% ProClin 300. |
| **Calibration Card:** | 1 |

**Warnings and Precautions**

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[5]

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

**Procedure**

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

**Calibration Details**

The Access Vitamin $B_{12}$ Calibrators are provided at six levels – zero and approximately 100, 250, 500, 900 and 1500 pg/mL – prepared gravimetrically from purified cyanocobalamin (vitamin $B_{12}$) and buffered matrix. Assay calibration data are valid up to 21 days.

Calibrators run in duplicate.

**Limitations of the Procedure**

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

# Access
Immunoassay Systems

# SAMPLE DILUENT A

## REF 81908 (Vial)

## REF A79783 (Diluent Pack)

**Intended Use**

The Access Sample Diluent A is intended for use with Access assays to dilute patient samples containing analyte concentrations greater than the analyte specific S5 calibrator.

**Summary and Explanation**

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

**Product Information**

**Access Sample Diluent A**

**Cat. No. 81908: 4 mL/vial**

- Provided ready to use.
- Allow the contents to stand for 10 minutes at room temperature.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

| | |
|---|---|
| **Diluent:** | Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin** 300. |

**Cat. No. A79783: 2 diluent packs, 32.9 mL/pack**

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 56 days after initial use of each well.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the diluent pack is damaged (i.e., broken elastomer), discard the pack.

| | |
|---|---|
| **R1a – R1e:** | Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300. |

**Warnings and Precautions**

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]

- Xi. Irritant: 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

## Procedure

Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the specific assay labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

## Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

# Access
Immunoassay Systems

# VITAMIN $B_{12}$ CALIBRATOR S0

## REF 33006

**Intended Use**

The Access Vitamin $B_{12}$ Calibrator S0 is intended for use with Access Vitamin $B_{12}$ to dilute patient samples containing analyte concentrations greater than the analyte specific S5 calibrator.

**Summary and Explanation**

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

**Product Information**

**Access Vitamin $B_{12}$ Calibrator S0**

**Cat. No. 33006: 4 mL/vial**

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.

| | |
|---|---|
| **S0:** | Buffered matrix with human serum albumin (HSA), < 0.1% sodium azide, and 0.25% ProClin** 300. Contains 0.0 pg/mL (pmol/L) vitamin $B_{12}$. |

**Warnings and Precautions**

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[5]
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]
- Xi. Irritant: 0.25% ProClin 300.

R 43: May cause sensitization by skin contact.

S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

**Procedure**

Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

**Limitations of the Procedure**

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

## References


1 Nelson DA and Davey FR. Erythrocytic disorders. In Clinical diagnosis and management by laboratory methods, 1991; 627-635. Edited by Henry JB, Philadelphia: W.B. Saunders Company.

2 Chanarin I. Megaloblastic anæmia. Cobalamin and folate. J. Clin. Pathol. 1987; 40: 978-984.

3 Hoffbrand AV. Vitamin $B_{12}$ and folate metabolism: the megaloblastic anæmias and other nutritional anæmias. In Blood and its disorders, 1982; 199-263. Edited by Hardistry RM and Weatherall DJ, Philadelphia: Blackwell Scientific Publications.

4 Herbert V. Folic acid and Vitamin $B_{12}$. In Modern nutrition in health and disease, 1973; 221-244. Edited by Goodhart RS and Shils ME, Philadelphia: Lea and Febiger.

5 HHS Publication, 4th ed., April 1999. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

6 DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.

7 Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.

8 Cembrowski GS, Carey RN. Laboratory quality management: QC⇌QA. ASCP Press, Chicago, IL, 1989.

9 Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

10 Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.

11 Chanarin I. The megaloblastic anaemias 3rd ed. Blackwell Scientific Publications, Oxford, 1990.

12 Taylor KB. Immune aspects of pernicious anaemia and atrophic gastritis. Clin Haematol 1976; 5: 497–519.

13 Tentative Guideline – User evaluation of precision performance of clinical chemistry devices, EP5-T. 1984. Clinical and Laboratory Standards Institute, 4: No. 8.

14 Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. Clinical Chemistry, 1984; 30: 290-292.





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