FOR PROFESSIONAL USE ONLY

Inhibin B Gen II CALIBRATORS AND CONTROLS

REF A81302

RUO FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

INTENDED USE

The Inhibin B Gen II Calibrators are intended to calibrate the Inhibin B Gen II ELISA assay A81301 for the quantitative measurement of inhibin B in serum and lithium heparin plasma. The Inhibin B Gen II Controls are intended for use as quality controls to monitor the precision of laboratory testing procedures for the A81301 Inhibin B Gen II ELISA.

SUMMARY AND EXPLANATION

Quantitative assay calibration is the process by which samples with known analyte concentration (i.e. assay calibrators) are tested in the same manner as patient samples to measure the response. The mathematical relationship between the measured response and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert absorbance measurements of patient samples to specific quantitative analyte concentration. Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of the Inhibin B Gen II ELISA assay. In addition, they are the integral part of good laboratory practice.[1] When performing the Inhibin B Gen II ELISA assay, include quality control materials to validate the integrity of the assay. The assayed values should fall within the acceptable range if the test system is working properly.

PRINCIPLE OF THE TEST

Two levels of controls are available to allow performance monitoring of the A81301 Inhibin B Gen II ELISA within the dynamic range. In the assay, calibrators, controls and samples are incubated in microtitration wells which have been coated with anti-activin B antibody.[2] After incubation and washing, the wells are incubated with biotinylated anti-Inhibin α-subunit detection antibody. After a second incubation and washing step, the wells are incubated with streptavidin labelled with the enzyme horseradish peroxidase (HRP). After a third incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). After incubation an acidic stopping solution is added. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as primary reference filter. The absorbance measured is directly proportional to the concentration of inhibin B in the samples. A set of Inhibin B Gen II calibrators is used to plot a calibration curve of absorbance versus inhibin concentration. The inhibin B concentrations in the samples can then be calculated from this calibration curve.

MATERIALS SUPPLIED

CAL A **Inhibin B Gen II Calibrator A:**
A95007

- Provided ready to use.
- One vial, 1.0 mL, labeled A, containing 0 pg/mL inhibin B in fetal bovine serum with < 0.5% ProClin* 300.
- Freeze upon receipt at -20°C. For multiple use, aliquot and freeze at -20°C.
- Stable until the expiration date stated on the label when stored at -20°C.
- Mix contents thoroughly by gentle inversion before use.
- After initial use, the thawed vials are stable at 2 to 8°C for seven days. Label the vials with the date of thaw.
- Return calibrator to 2 to 8°C after each use.
- Thaw calibrator no more than two times.
- Signs of possible deterioration are control values out of range.

CAL B-G **Inhibin B Gen II Calibrator B-G:**
A95008, A95009, A95010, A95011, A95012, A95013

- Provided ready to use.
- Six vials, 1.0 mL, labeled B-G, containing concentrations of approximately 10, 30, 100, 250, 500 and 1000 pg/mL inhibin B in fetal bovine serum with < 0.5% ProClin 300.
- Freeze upon receipt at -20°C. For multiple use, aliquot and freeze at -20°C.
- Stable until the expiration date stated on the label when stored at -20°C.
- Mix contents thoroughly by gentle inversion before use.
- After initial use, the thawed vials are stable at 2 to 8°C for seven days. Label the vials with the date of thaw.
- Return calibrators to 2 to 8°C after each use.
- Thaw calibrators no more than two times.
- Signs of possible deterioration are control values out of range.
- Refer to vial labels for exact concentration.

CTRL I-II **Inhibin B Gen II Controls:**
A95014, A95015

- Provided ready to use.
- Two vials, 1.0 mL, labeled I and II, containing low and high concentrations of inhibin B in buffer with protein (bovine), < 0.5% ProClin 300, and sodium azide.
- Freeze upon receipt at -20°C. For multiple use, aliquot and freeze at -20°C.
- Stable until the expiration date stated on the label when stored at -20°C.
- Mix contents thoroughly by gentle inversion before use.
- After initial use, the thawed vials are stable at 2 to 8°C for seven days. Label the vials with the date of thaw.
- Return controls to 2 to 8°C after each use.
- Thaw controls no more than two times.
- Signs of possible deterioration are control values out of range.
- Refer to vial labels for exact concentration.

TRACEABILITY

The Inhibin B Gen II Calibrators are traceable to the World Health Organization Reference Reagent for Inhibin-B (Code 96/784) from NIBSC and performance verified by immunoassay. The assigned values were established using representative samples from this lot of calibrators and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. **Inhibin B Gen II Reagents A81301**
2. Microtitration plate reader capable of absorbance measurement at 450/405 nm and preferentially capable of dual wavelength (reference filter) at 600 to 630 nm
3. Deionized water
4. Precision pipette to deliver 10–1000 µL
5. Microtitration plate shaker capable of 600–800 orbital revolutions per minute (rpm)
6. Microtitration plate washer
7. Vortex mixer
8. Absorbent materials for blotting the strips
9. Graph paper for manual data reduction

QUALITY CONTROL VALUE ASSIGNMENT

- The control values were derived by analyzing control replicates run in the A81301 assay, and are lot-specific.
- Individual laboratory means may vary from the listed values, but should fall within the corresponding acceptable range.
- Variation between laboratories may be caused by differences in laboratory technique, assay incubation temperature and instrumentation.
- It is recommended that each laboratory establish its own mean and acceptable range values and use those provided only as guides.

WARNINGS AND PRECAUTIONS

- **For Research Use Only**. **Not for use in diagnostic procedures.**
- Use good laboratory practices.[1]
- 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.[3] Use an appropriate disinfectant for decontamination. Store and dispose of

these materials and their containers in accordance with local regulations and guidelines.

- Inhibin B Gen II Calibrator A / Sample Diluent



WARNING
H317 May cause an allergic skin reaction.
P280 Wear protective gloves, protective clothing and eye/face protection.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Inhibin B Gen II Cal B, C, D, E , F, G



WARNING
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P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Inhibin B Gen II Control I & II



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P362+P364 Take off contaminated clothing and wash it before use.
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Xi: Irritant: < 0.5% ProClin 300.



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

- SDS Safety Data Sheet is available at techdocs.beckmancoulter.com

CALIBRATION DETAIL

The Inhibin B Gen II calibrators are provided at seven levels —zero and approximately 10, 30, 100, 250, 500 and 1000 pg/mL. Run the Inhibin B Gen II Calibrators A-G and Control Levels I and II in duplicate.

CURVE DETAIL

1. Calculate the mean absorbance for each calibrator, control or sample.
2. Subtract the mean absorbance of Calibrator A (blank) from the mean absorbance of calibrators B-G, controls and samples. Plot the log of the mean blank-subtracted absorbance readings for calibrators B-G along the y-axis versus log of the inhibin B concentrations in pg/mL along the x-axis, using a cubic regression curve-fit. Alternatively, the data can be plotted log vs. log and a linear curve-fit can be used.
3. Determine the inhibin B concentrations of the controls and samples from the calibration curve by matching their mean absorbance readings with the corresponding inhibin B concentrations.

LIMITATIONS

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

REFERENCES

1 Clinical Laboratory Safety; Approved Guideline Third Edition, GP17-A3. 2012. Clinical and Laboratory Standards Institute.

2 Ludlow H, Muttukrishna S, Hyvönen M, Groome, NP. 2008. Development of a new antibody to the human inhibin/activin betaB subunit and its application to improved inhibin B ELISAs. J Immunol Methods 329:102-111.

3 HHS Publication, 5th ed., December 2009. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5

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Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)



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