accu-beads®

3 M/ml – 18 M/ml – 35 M/ml

A Quality Control Check for Automated and Manual Sperm Counting Methods

Instructions for Use for the following Hamilton Thorne, Inc. part items:

Ref	Volume (per bottle)	Concentration
710259	5 ml	3 M/ml
710257	5 ml	18 M/ml
710258	5 ml	35 M/ml
710110	5 ml	18 M/ml, 35 M/ml
710111	5 ml	3 M/ml, 18 M/ml, 35 M/ml

⚠ *Reseal tightly after each use to prevent evaporation of solution. Prolonged openings may affect bead concentrations.*

Intended Use:

Accu-beads® are intended for use as a quality control to assist in verifying the accuracy of sperm counting procedures performed either manually, using a microscope, or automatically, using a computer-assisted sperm analysis (CASA) system. Accu-beads are not intended as a quality control for determining motility accuracy.

Reagents: For in-vitro diagnostic use only.

Summary and Principle:

The accu-beads® solution consists of small latex spheres (4 µm in diameter) suspended in an isopycnic medium. The physical properties of this medium allow the beads to float and promote even distribution throughout the solution. Each bottle contains a 5 ml latex bead suspension.

Accu-beads is designed primarily for use with capillary-loading chambers for verification of automated sperm counting methods.

For CASA counting methods, accu-beads verify the performance of the optical system and computer image processing. For manual counting methods, accu-beads verifies technician techniques and counting practices. Under both circumstances, the precision of the chamber used is also verified.

An accu-beads quality control check is recommended each day prior to the evaluation of actual sperm samples.

Storage and Stability:

Store at room temperature.
Shelf Life: 24 months from date of manufacture.
Product can be used safely for duration of shelf life, when stored at room temperature and aseptic conditions are maintained.

Warning:

⚠ *Contains 0.07% Sodium Azide, avoid contact with eyes.*

Instructions for Use:

All laboratory procedures described herein are recommendations only. Each laboratory should establish and validate its own procedures for preparation and use.

Preparation of Accu-beads®

1. Vortex or shake the accu-beads® bottles thoroughly for 30-45 seconds to promote even distribution of the beads throughout the solution.
2. The accu-beads should be used in the same manner which would normally be used for standard sperm counts (e.g., volume, dilution, counting method).
3. Use Immediately after shaking, and pipette the required volume (follow the chamber manufacturer's instructions) of bead solution into the counting chamber.
4. Place the counting chamber on the analyzer or microscope stage.

Acceptable Accu-beads Count Range

Fixed Coverslip Chambers

35 M/ml: ± 5 M/ml (30 - 40 M/ml)
18 M/ml: ± 2.5 M/ml (15.5 - 20.5 M/ml)
3 M/ml: ± 1 M/ml (2.0 - 4.0 M/ml) *

⚠ Verification of bead concentrations should only be performed using a hemocytometer. Follow steps 1 and 2 above for the Preparation of Accu-beads, and then pipette 100 µl of accu-beads into a test tube. Add 1900 µl of diH2O and agitate. Pipette the mixture into the hemocytometer.

*Hemacytometer***

46 M/ml: ± 7 M/ml (39 - 53 M/ml)
23 M/ml: ± 4 M/ml (19 - 27 M/ml)
4 M/ml: ± 1.5 M/ml (2.5 - 5.5 M/ml) *

** Concentrations < 18 M/ml are intended for Human Clinical QC applications only and not necessary in non-clinical settings.*
*** Calculations based on effects of viscosity and hemacytometer flow dynamics as described in accu-beads Research Report 45. Contact Hamilton Thorne for more information.*

Manual Counting Procedures

1. Count the beads according to a standard counting procedure.
2. When using an eyepiece reticle, at least 10 squares in 5 different fields should be counted. The number of beads counted should be at least a minimum 200. For a higher degree of accuracy, count more fields.
3. When using a fixed chamber with a gridded coverslip or a gridded slide, follow the chamber manufacturer's counting instructions.
4. Calculate the bead concentration according to the chamber manufacturer's instructions.
5. Count another aliquot of the same sample. The results should be within 10% of each other to be considered valid.
6. If the results are valid, average the two counts and compare to the accu-beads® acceptable ranges listed above.
7. The counting procedure above should be performed with all accu-beads® concentrations.
8. Record all results along with pertinent information such as the chamber used and the name of the person performing the QC procedure.

Troubleshooting for Manual Counting Procedures

If concentration is too high or too low:

1. Mixing Error:
   a. Mix accu-beads® again for even distribution.
2. Pipetting Error:
   b. Reload the chamber being careful to avoid overloading or underloading the chamber.
3. Technician Error:
   c. Have a different technician confirm counting accuracy.
4. Chamber Error:
   d. Repeat the QC procedure using another chamber of known depth.

Automated Counting Procedures

Using Accu-beads® for QC of Computer-Assisted Sperm Analyzers

Do not dilute the accu-beads. Diluting may cause sampling errors.

Preparation of HT CASA II Software

1. From the **MAIN** screen, select **SETUP**.
2. Create or select the accu-beads setup.
3. Set the parameter settings in accordance with the guidelines in the chart below.
4. Ensure that the appropriate chamber and depth have been selected.
5. Make sure that the 10x NH phase contrast objective is in place.
6. Verify the proper **MAGNIFICATION**.
7. Perform a **LIVE CALIBRATION** and set the **ILLUMINATION**. The accu-beads should be illuminated blue.

CASA II SYSTEM Settings	Version 1.09 & Higher
Frame Count	5
Frame Capture Speed (Hz)	60
Elongation Max	100
Elongation Min	
Head Brightness Max	150
Head Size Max	150
Head Size Min	10
Static Require Tails	No
Capillary Correction	

Preparation of Legacy CASA (IVOS & CEROS) Software Version 10

1. From the **MAIN MENU,** select **SETUP**.
2. Choose an **ANALYSIS SETUP** that is not in use by your laboratory for other studies, and name it accu-beads (or something similar). Since the accu-beads will be used on a regular basis, it is best to designate one **ANALYSIS SETUP** solely for the QC process.
3. Set the parameter settings in accordance with the guidelines in the chart below.
4. Press **CONFIGURE STAGE**. Select the appropriate chamber and depth.
5. Make sure that the 10x NH phase contrast objective is in place.
6. Press **CALIBRATE OPTICS**. Verify the proper **MAGNIFICATION**. Set **ILLUMINATION** for a **LOW PHOTOMETER** reading of 50 - 55.

Preparation of Legacy CASA (IVOS & CEROS) Software Version 12 & 14

1. From the **MAIN MENU**, select **SETUP**.
2. Choose an **ANALYSIS SETUP** that is not in use by your laboratory for other studies and name it accu-beads (or something similar). Since the accu-beads will be used on a regular basis, it is best to designate one **ANALYSIS SETUP** solely for the QC process.
3. Set the parameter settings in accordance with the guidelines in the chart below.
4. Press **STAGE SETUP**. Select the appropriate chamber and depth.
5. Make sure that the 10x NH phase contrast objective is in place.
6. Press **OPTICS SETUP**. Verify the proper **MAGNIFICATION**. Set **ILLUMINATION** for a **PHOTOMETER** reading of 50 - 55.

LEGACY CASA (IVOS & CEROS) SYSTEMS	Versions 10, 12, 14
Frames/ No. of Frames	5
Frame Rate / Frames /sec	60
Minimum Contrast	50
Minimum Size	3
Non-motile Head Size / Cell Size	5
Non-motile Intensity / Cell Intensity	140
Size Limits or Gates	0.5 to 8.0
Intensity Limits or Gates	0.5 to 2.0
Elongation Limits	20 to 100

Performing Accu-beads® Analysis (All CASA systems)

1. **Focus** the image clearly.
2. Select a single field for analysis and only analyze one field at a time.
3. When analysis is complete, view the **PLAYBACK** screen.
4. Check that nearly all beads are labeled properly.
5. If optimum labeling is not achieved, refer to the analyzer manual for QC procedures on the identification of non-Motile cells.
6. When the labeling is satisfactory, count, at a minimum, 200 cells for analysis. Record, print or store the concentration results.
7. Perform a second analysis from an aliquot of the same sample. The results should be within 10% of each other to be considered valid.
8. If the results are valid, average the two counts and compare to the Acceptable accu-beads Count Ranges.
9. The QC procedure above should be performed with all concentrations of beads.

Troubleshooting for Automated Counting Procedures

1. If concentration is too high or too low:
   a. Check the dilution factor on the INFO screen.
   b. Check the Magnification setting.
   c. Review PLAYBACK to check proper labeling.
   d. Check/adjust analysis parameters as described in the manual.
   e. Repeat analysis with new chamber.
   f. See also: Troubleshooting for Manual Counting Procedures.
2. Motility is detected:
   a. Wipe excess fluid off chamber, wait 1 minute and reanalyze.
   b. Stage movement may cause slight movement of beads. To prevent this, use the ADD SCAN feature.
   c. CASA II: Adjust Slow and Static VAPN SL.
   d. Legacy Systems: Set Slow Cells to Static or Non-motile.
3. Image is poor:
   a. Adjust focus.
   b. Check ILLUMINATION.
   c. Ensure that the 10 NH phase contrast objective is in place.

Product Specifications:

A Certificate of Analysis is available for each batch upon request from our website with respective lot number.

The Safety Data Sheet for accu-beads is available upon request and can also be downloaded from our website.

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