NanoEntek

CE IVD

# ADAMII™-CD34 Kit

Hematopoietic Stem Cell Counting System

REF CD34K-025

IVD For *in vitro* diagnostic use only

## Intended use

The ADAMII™ CD34 System includes the ADAMII™-CD34 Kit which is designed for use with the ADAMII™ instrument, a benchtop image-based fluorescence cell counter. ADAMII™ CD34 System provides enumeration of viable CD34+ cells, viable CD45+ cells, and calculates percentage of viable CD34+ cells out of viable CD45+ cells. ADAMII™ CD34 System can be used for mobilized peripheral blood (MPB) collected in Na-Heparin or EDTA, haematopoietic progenitor cell – apheresis (HPC-A) collected in ACD or ACD+Heparin, fresh cord blood (FCB) collected in CPD, and thawed frozen cord blood (TFCB) collected in CPD and stored with 10% DMSO, 1% Dextran 40. ADAMII™ CD34 System is intended for use in clinical laboratories and for in vitro diagnostic use only. It is not intended for use in point-of-care settings.

## Summary and explanation of test

Myeloablative and non-myeloablative allogenic stem cell transplants are curative options for many patients with hematologic malignancy. The number of both autologous and allogeneic transplants has been steadily rising over the last two decades. Mobilized peripheral blood (MPB) stem cells and hematopoietic progenitor cell- apheresis (HPC-A) are used as a preferred source of stem cells for autologous and allogeneic hematopoietic stem cell transplantation.[1,2] Umbilical cord blood (CB) has been an alternative hematopoietic stem cell source, especially for patients without an appropriate marrow or mobilized peripheral blood donor.[3]

CD34 antigen, a single chain transmembrane glycoprotein expressed on primitive blood and bone marrow-derived progenitor cells, is a well-known marker for hematopoietic and endothelial progenitors.[4] Accurate measurement of CD34+ cell number is very important in clinical practice as hematopoietic transplantation protocols have specific dose requirements and the effect of mobilizing agents may be different in normal donors and cancer patients.[5,6,7] Flow cytometric determination of CD34+ cells has rapidly become the tool of choice for quantitating circulating hematopoietic progenitors, for establishing their minimum number to ensure the engraftment and the optimal timing of apheresis.[8,9,10]

Despite the reliability of flow cytometric assay, inter-laboratory variation has been reported with flow cytometric methods for determining the percentage and absolute numbers of CD34+ cells.[11]
ADAMII™ CD34 Stem Cell Counting System provides accurate and precise CD34+ cell counts, and ratios of CD34+ and CD45+ cells with rapid turnaround and minimal input from an operator.[12] High degree of precision of ADAMII™ CD34 Stem Cell Counting System is due in part to simplified sample preparation steps, and elimination of washing steps, which may cause cell loss and could be prone to human error. In addition, ADAMII™ CD34 Stem Cell Counting System uses a customized software which does not require post-measurement interpretation steps.

## Principle of the assay

ADAMII™-CD34 assay starts with adding an appropriate volume of a sample to a test tube and mixing with reagent containing fluorescence-labeled antibodies and nucleic acid staining dye. Fluorescence-labeled antibodies bind specifically to CD34 and/or CD45 markers expressed on cell surfaces. Nucleic acid staining dye binds specifically to nucleus of dead cells. In ADAMII™-CD34 Kit, CD34 antibodies (clone 8G12) recognize CD34 marker expressed on hematopoietic stem cells, CD45 antibodies (clone 2D1) recognize CD45 marker expressed on leukocytes.

After a sample is incubated with reagent for 20 minutes, RBC Lysis Buffer is added to lyse red blood cells. After completion of RBC lysis, sample preparation is complete and is ready to be measured. Prepared sample is loaded into a disposable plastic slide and loaded slide is placed on precision stage in ADAMII™ instrument.

In ADAMII™ CD34 software, user is expected to adjust focusing using either manual focusing buttons or autofocusing button. After good focuses have been found, user presses "Run Sample" button to start image acquisition. While images are being taken, ADAMII™ CD34 software analyzes images to produce measurement results. After completion of image acquisition, final results will be shown on screen and automatically saved.

Final results include (1) Viable CD34+ cells/μL, (2) Viable CD45+ cells/μL, (3) Total CD34+ cells/μL, (4) Total CD45+ cells/μL, (5) CD34 Viability (%), (6) CD45 Viability (%), (7) the ratio of Viable CD34+ out of Viable CD45 (%).

CD34 antibody recognizes a 105-120-kilodalton (kDa) single-chain transmembrane glycoprotein. Clone 8G12 recognizes an epitope on CD34 distinct from the one recognized by clone My10; at least three epitopes have been identified. CD34 antibody (clone 8G12) is composed of mouse IgG1 heavy chains and kappa light chains. Excitation is at 496nm/ Emission: 578nm.

## Material provided

| Q'ty | Contents | Catalogue number |
|---|---|---|
| 1 | ADAMII™ CD34 Reagent (25 Tests) | A34R-001 |
| 1 | ADAMII™ 10X RBC Lysis Buffer (4 mL) | A34L-001 |
| 1 | ADAMII™ Calibration Beads (25 Tests) | A2CB-001 |
| 1 | ADAMII™ Assay Slide (a pack of 25 slides) | A2AS-025 |
| 1 | ADAMII™ CD34-Kit Package Insert | - |
| 1 | ADAMII™ 10X RBC Lysis Buffer (for Control Material) | ACL-001 |

⚠ ***Note:*** *10X RBC lysis buffer for control material is offered separately in an aluminum pouch.*

**One Reagent solution CD34 Reagent contains:**

PE-conjugated anti-CD34 antibody

PerCP-conjugated anti-CD45 antibody

Nucleic acid staining dye

## Materials required but not provided

- Reagent-grade (deionized) water
- EDTA blood collection tubes or equivalent
- Microcentrifuge tube
- 1X PBS (without Calcium and Magnesium) if sample dilution is necessary
- Pipettes and pipette tips (5μL, 20 μL, 35 μL, 100 μL, 250 μL, 1,000 μL)
- Vortex mixer
- Timer
- Ice bucket filled with shredded ice if a refrigerator is not available nearby
- Personal protective equipment
- Biohazard waste disposal containers
- Control material

## Warning and Precautions

- For in vitro diagnostic use only
- Do not use the reagent or Assay Slide if you observe any change in appearance.
- Do not decontaminate ammonium chloride lysed samples with bleach.
- To achieve accurate results, it is critical to add a precise volume of the specimen to an empty tube and mix it with a precise volume of CD34 reagent solution containing antibodies and nucleic acid stains.

  **Use the method of reverse pipetting or a positive displacement pipette to aliquot samples. See the pipette manufacturer's instructions for more information.*
- Ammonium chloride lysing solution is harmful if swallowed (R22) and irritating to the eyes (R36). Wear suitable protective clothing, eyewear, and gloves. Dispose of in accordance with federal, state, and local regulations.
- All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection. Handle as if capable of transmitting infection and dispose of it with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth.
- When using lysis buffer, use after dilution.
- After using Calibration Beads, close the cap tightly.

## Storage and Stability

All unopened/opened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for 12 months from the date of manufacture. The expiration date is clearly indicated on the product box, pouch, tube, and bottle.

| Material | Catalogue number |
|---|---|
| Refrigerator temperature storage (2~8 ℃) | |
| ADAMII™ CD34 Reagent | A34R-001 |
| ADAMII™ Calibration Beads | A2CB-001 |
| ADAMII™ 10X RBC Lysis buffer (4 mL) | A34L-001 |
| ADAMII™ 10X RBC Lysis Buffer (for Control Material) | ACL-001 |
| | |
| Ambient temperature storage (2~25 ℃) | |
| ADAMII Assay Slide | A2AS-025 |

## Specimen collection and handling

- Keep undiluted specimens stored at 2~8°C.
- Laboratory should be validated the pre-analytical sample with the storage conditions.
- Stain fresh specimens (MPB and HPC-A) within 24 hours of collection. Stain fresh cord blood within 48 hours of collection. Stain frozen specimens immediately after thawing.
- Do not use previously fixed samples.
- Do not use fresh MPB or HPC-A samples that have been stored for more than 24 hours.
- Do not use fresh cord blood samples that have been stored for more than 48 hours.
- Reject clotted, or clumped specimens.
- After completion of RBC lysis, store prepared samples on ice. Fresh MPB, fresh HPC-A and fresh cord blood samples can be measured within 1hr after completion of RBC lysis. Frozen specimens should be measured immediately after completion of RBC lysis.
- Before staining for CD34 counting in ADAMII™, HPC-A products (collected in ACD or ACD+Heparin), MPB samples (collected in Na-Heparin), and fresh cord blood samples (collected in CPD) and thawed frozen cord blood samples need to be transferred into EDTA anticoagulant tubes to reduce cell clumping.
- HPC-A sample: Before starting a sample preparation, adjust the number of total white blood cells (WBC) in a sample to be below 150,000 cells/μL by diluting the sample with 1X PBS.
- MPB sample: After completion of RBC lysis, further dilute a prepared sample if the number of total white blood cells (WBC) in the original sample is over 35,000 cells/μL by diluting the prepared sample with 1X RBC Lysis buffer.

# Procedure

## Calibration

The Calibration Beads for the ADAMII™ instrument is included in the kit are fluorescent particles with specific sizes and fluorescence dyes, which enable users to check if the ADAMII™ instrument is in good condition in terms of optic alignment and software algorithm to count cells by processing images from multiple different channels. It is recommended that the calibration is done regularly or at least once a week. Refer to ADAMII™ Instrument User Manual for complete instructions.

## Quality control

In accordance with the Good Laboratory Practice and laboratory regulations, it is recommended to run two levels of quality control materials (procedural control) to monitor the performance of entire analytic processes.

Each laboratory should establish its own practice to run quality controls. Each laboratory may determine when or how often to run quality controls in consideration of followings:

(1) at least once a month
(2) when receiving ADAMII™ CD34 kits from a new lot
(3) when there is new operator
(4) when problems in ADAMII™ CD34 instrument or kits are identified
(5) when problems during shipping or delivery of ADAMII™ CD34 kits are identified
(6) when it is required by laboratory's standard QC procedures.

Commercial controls provide established values for absolute counts of CD34+ cells and percentage of CD34+ cells out of CD45+ cells. Commercial controls are available from a few manufacturers. Streck CD-Chex CD34 controls have been tested extensively with ADAMII™ CD34.

**1. Quality control procedure**

(1) Label an empty tube and the ADAMII™ Assay Slide for the sample identification.

(2) Transfer 20 µL of well-mixed control material to the empty tube and add 5 µL of ADAMII™ CD34 Reagent solution. Then, cap the tube and mix well by vortexing or finger flicking.

⚠ ***Note:*** *Vortex the ADAMII™ CD34 Reagent before use.*

(3) Incubate for 20 minutes in the dark at room temperature (66~77°F, 20~25°C).

(4) Dilute ADAMII™ 10x RBC lysis buffer (for control material) that is provided separately to a new empty tube to prepare 1x RBC lysis buffer.

⚠ ***Note:*** *1x lysis buffer should be prepared enough for use each day. Prepare by diluting 1 part of 10x lysis buffer with 9 parts of distilled water. Store and use at room temperature (66~77°F, 20~25°C)*

(5) Add 35 µL of 1x RBC lysis buffer for control material to the tube and vortex for 2-3 seconds.

(6) Incubate for 10 minutes in the dark at room temperature (66~77°F, 20~25°C).

(7) Load 25 µL of the stained control material onto the ADAMII™ Assay Slide.

⚠ ***Caution:*** *Make sure to add fluid slowly. Fast injection may cause spill-over.*

(8) Wait 3 minutes for the sample to settle.

(9) Insert the sample loaded ADAMII™ Assay Slide into the ADAMII™ instrument and select 'Control' for sample type.

(10) Please refer to ADAMII™ user manual for running measurements.

## Specimen processing

### 1. Preparation

- HPC-A/MPB

(1) Label an empty tube and an ADAMII™ Assay Slide for the sample identification.

(2) Transfer 20 µL of a well-mixed specimen to the empty tube and add 5 µL of ADAMII™ CD34 Reagent solution. Then, cap the tube and mix well by vortexing or finger flicking.

⚠ ***Note:*** *Vortex the ADAMII™ CD34 Reagent before use.*

(3) Incubate for 20 minutes in the dark at room temperature (66~77°F, 20~25°C).

(4) Dilute ADAMII™ 10x RBC lysis buffer that is included in the kit to a new empty tube to prepare 1x RBC lysis buffer.

⚠ ***Note:*** *1x lysis buffer should be prepared enough for use each day. Prepare by diluting 1 part of 10x lysis buffer with 9 parts of distilled water. Store and use at room temperature (66~77°F, 20~25°C)*

(5) Add 35 µL (for MPB sample) or 250 µL (for HPC-A) of 1X RBC Lysis buffer to the tube and vortex for 2-3 seconds.

(6) Incubate for 10 minutes in the dark at room temperature (66~77°F, 20~25°C).

- Fresh Cord Blood / Frozen Cord Blood specimen

(1) Label an empty tube and an ADAMII™ Assay Slide.

(2) Transfer 50 µL of a well-mixed specimen to the empty tube and add 5 µL of ADAMII™ CD34 Reagent solution. Then, cap the tube and mix well by vortexing or finger flicking.

⚠ ***Note:*** *Vortex the ADAMII™ CD34 Reagent before use.*

(3) Incubate for 20 minutes in a dark space at room temperature (66~77°F, 20~25°C).

(4) Dilute ADAMII™ 10x RBC lysis buffer that is included in the kit to a new empty tube to prepare 1x RBC lysis buffer.

⚠ ***Note:*** *1x RBC lysis buffer should be prepared enough for use each day. Prepare by diluting 1 part of 10x RBC lysis buffer with 9 parts of ultrapure water. Store and use at room temperature (66~77°F, 20~25°C)*

(5) Add 200 µL of 1X RBC Lysis buffer to the tube and vortex for 2-3 seconds.

(6) Incubate for 10 minutes in a dark space at room temperature (66~77°F, 20~25°C).

| Sample type | Sample (µL) | ADAMII-CD34 reagent (µL) | RBC Lysis buffer (µL) |
|---|---|---|---|
| **Control material** | 20 | 5 | 35 (for control material) |
| **MPB** | 20 | 5 | 35 |
| **HPC-A** | 20 | 5 | 250 |
| **Cord blood (FCB, TFCB)** | 50 | 5 | 200 |

**2. Assay procedure**

(1) Load 25 µL of the stained sample onto the ADAMII™ Assay Slide.

⚠ ***Caution:*** *Vortex the cells thoroughly, at low speed, to mix cells well and to reduce the number of aggregations before loading stained cells on an Assay slide.*

⚠ ***Caution:*** *Make sure to add fluid slowly. Fast injection may cause spill-over.*

(2) Wait 3 minutes for the sample to settle.

(3) Insert the loaded ADAMII™ Assay Slide into ADAMII™ instrument and select a corresponding sample type.

(4) Please refer to ADAMII™ user manual for running measurements.

## Procedural notes

- To minimize errors and variations during sample preparation, it is highly recommended to use reverse pipetting technique.
- It is highly recommended to mix samples, reagents, and test tubes well at every steps.
- Laboratory must establish their own CD34+ viability requirements for each specimen type.
- Avoid bubbles at all times of sample loading onto the slide for an accurate result.
- Erroneous measurements can be made if tubes are exposed to bright lights or sun lights.
- Erroneous measurements can be made if prepared samples are not stored on ice after completion of RBC lysis, or if prepared samples have been kept for more than one hour before being measured.
- Fixed and stored specimens should be avoided. Avoid using hemolyzed, clotted, or clumped specimens.
- Avoid using hemolyzed, clotted, or clumped specimens.

## Calculation of results

ADAMII™ instrument performs all image acquisition and analysis automatically. Measurement results include (1) Viable CD34+ counts (cells/µL), (2) Viable CD45+ counts (cells/µL), (3) Total CD34+ counts (cells/µL), (4) Total CD45+ counts (cells/µL), (5) the ratio of Viable CD34 out of Viable CD45 (%), (6) CD34 Viability (%), and (7) CD45 Viability (%).

**Detection range**

The detection range is as follows:

CD34: 1~1000 cells/µL

# Performance characteristics

## Method comparison

Viable CD34+ counts [cells/μL], viable CD45+ counts [1000 cells/μL], and the ratio of Viable CD34 out of Viable CD45 [%] were measured using ADAMII™-CD34 Kits for mobilized peripheral blood samples (MPB collected in EDTA or Na-Heparin), leukapheresis samples (HPC-A collected in ACD or ACD+Heparin), fresh cord blood (FCB collected in CPD) samples, and thawed frozen cord blood (TFCB collected in CPD and stored with 10% DMSO and 1% Dextran 40) samples. These results were compared to a predicate assay (BD Stem Cell Enumeration Kit used in FACSCalibur or FACSLyric). Result from two methods were compared using regression analysis (slope, intercept and $R^2$) and 95% confidence intervals.

⚠ ***Note:*** *ADAMII™-CD34 Kits have been qualified for these sample types; MPB collected in EDTA or Na-Heparin, HPC-A collected in ACD or ACD+Heparin, FCB collected in CPD, and TFCB collected in CPD and stored with 10% DMSO and 1% Dextran 40.*

### Regression Analysis

**Table 1. Regression Statistics on ADAMII™ with ADAMII™ CD34 Kit**

| | N | $R^2$ | Slope/95% CI | Intercept/95% CI |
|---|---|---|---|---|
| **MPB (pooled)** | | | | |
| Viable CD34 (cells/μL) | 248 | 0.99 | 0.997 (0.985 - 1.009) | 0.317 (-0.017 - 0.641) |
| Ratio of viable CD34 in viable CD45 | 248 | 0.99 | 1.000 (0.968 - 1.000) | 0 (0 – 0.003) |
| Viable CD45 (1000 cells/μL) | 248 | 0.99 | 1.018 (1.008 - 1.030) | 0.111 (-0.072 - 0.249) |
| **HPC-A (pooled)** | | | | |
| Viable CD34 (cells/μL) | 382 | 0.99 | 0.998 (0.989 - 1.007) | 2.237 (-2.033 - 6.029) |
| Ratio of viable CD34 in viable CD45 | 382 | 0.98 | 1.000 (0.983 - 1.007) | 0.010 (0.006 – 0.013) |
| Viable CD45 (1000 cells/μL) | 382 | 0.99 | 0.982 (0.972 – 0.992) | 0.760 (0 - 1.495) |
| **Fresh cord blood (pooled)** | | | | |
| Viable CD34 (cells/μL) | 124 | 0.99 | 0.994 (0.980 – 1.009) | -0.115 (-0.725 – 0.316) |
| Ratio of viable CD34 in viable CD45 | 124 | 0.99 | 1.033 (1.013 – 1.056) | -0.02 (-0.03 – -0.01) |
| Viable CD45 (1000 cells/μL) | 124 | 0.98 | 0.945 (0.919 – 0.968) | 0.222 (0.067 – 0.401) |
| **Thawed frozen cord blood (pooled)** | | | | |
| Viable CD34 (cells/μL) | 159 | 0.99 | 0.983 (0.964- 1.001) | 0.519 (-0.080 – 1.210) |
| Ratio of viable CD34 in viable CD45 | 159 | 0.96 | 0.995 (0.959 – 1.029) | -0.01 (-0.02 – 0.01) |
| Viable CD45 (1000 cells/μL) | 159 | 0.95 | 0.993 (0.962 – 1.023) | 0.203 (-0.029 – 0.571) |

## Regression Plots

**Figure 1. Pooled data CD34 cells/µL**

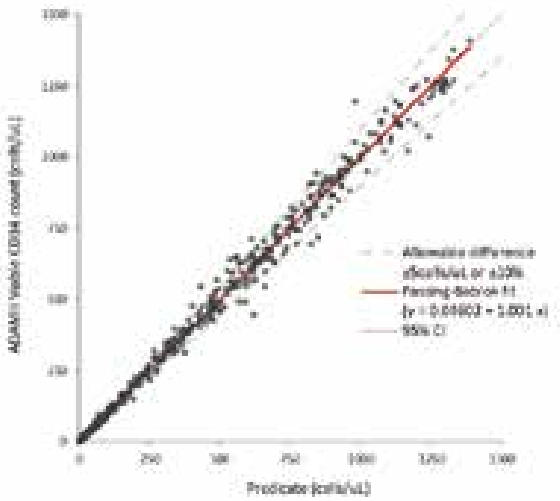


**Figure 2. Pooled data %CD34 of CD45**

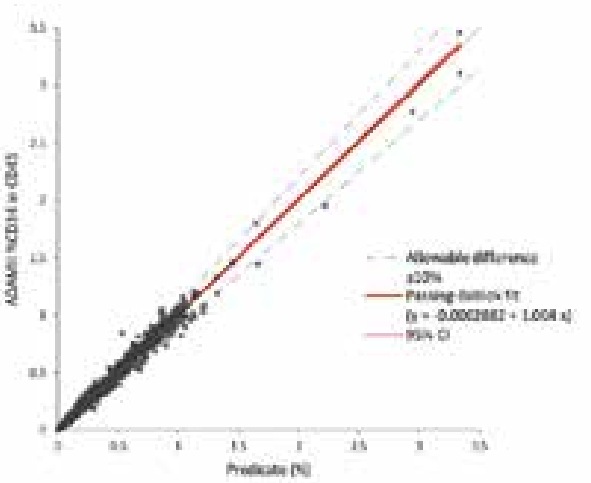
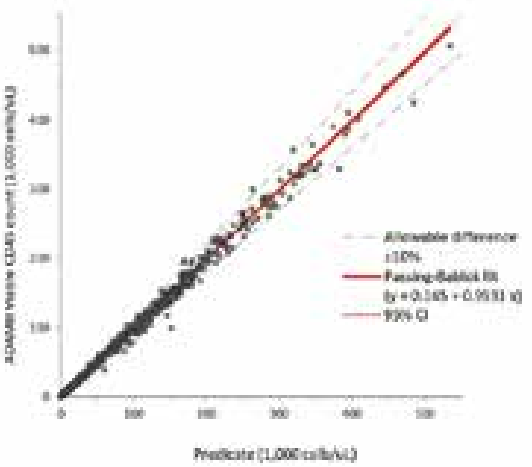


**Figure 3. Pooled data CD45 (1000 cells/µL)**



## Precision

- **Study 1**

Estimates of assay precision were assessed at the NanoEntek Research laboratory using two levels of control material with ranges of:

- Med : 22.3 < CD34+ counts (cells/μL) ≤ 36.3
- High : 86.8 < CD34+ counts (cells/μL) ≤ 126.8

Two replicates on two separate runs for 20 days were assessed for Repeatability (within-run), between-run, between-day, and within-laboratory precision.

**Table 2. Study 1 Results**

| CD34 Count Mean (cells/μL) | Repeatability | | Between-Run | | Between-Day | | Within-Laboratory | |
|---|---|---|---|---|---|---|---|---|
| | SD | CV | SD | CV | SD | CV | SD | CV |
| 31.814 | 2.987 | 9.4% | 0.000 | 0.0% | 2.187 | 6.9% | 3.702 | 11.7% |
| 106.220 | 5.557 | 5.2% | 0.000 | 0.0% | 6.537 | 6.2% | 8.580 | 8.1% |

- **Study 2**

An additional single site study was conducted at the NanoEntek Research laboratory using a lower control CD34+ count range: 9.7 < CD34+ counts (cells/μL) ≤ 17.7). Two replicates on two separate runs per day for 21 days were assessed.

**Table 3. Study 2 Results**

| CD34 Count Mean(cells/ μL) | Repeatability | | Between-Run | | Between-Day | | Within-Laboratory | |
|---|---|---|---|---|---|---|---|---|
| | SD | CV | SD | CV | SD | CV | SD | CV |
| 12.615 | 1.620 | 12.8% | 1.008 | 8.0% | 0.005 | 2.9% | 1.942 | 15.4% |
| %CD34 of CD45 Mean | Repeatability | | Between-Run | | Between-Day | | Within-Laboratory | |
| | SD | CV | SD | CV | SD | CV | SD | CV |
| 0.181 | 0.025 | 13.6% | 0.016 | 8.6% | 0.005 | 2.9% | 0.030 | 16.4% |
| CD45 Count Mean(cells/ μL) | Repeatability | | Between-Run | | Between-Day | | Within-Laboratory | |
| | SD | CV | SD | CV | SD | CV | SD | CV |
| 6985.959 | 224.878 | 3.2% | 140.769 | 2.0% | 140.306 | 2.0% | 300.120 | 4.3% |

- **Study 3**

An additional single-site study was conducted at the NanoEntek Research laboratory using 3 lots of reagents, 3 operators, and 3 instruments using 3 levels of control.

**Table 4. Study 3 Results**

| | CD34 cells/μL | CD34 % | CD45 cells/μL |
|---|---|---|---|
| Low | 9.2-17.2 | 0.13-0.27 | 5600-7600 |
| Med | 25.6-39.6 | 0.39-0.59 | 5700-7700 |
| High | 92.2-132.2 | 1.32-1.92 | 5900-7900 |

**Table 5. Study 3 Results by Instrument**

| Instrument to Instrument | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Mean Value | N | Repeatability | | Between- Days | | Between-Run | | Between-Instrument | | Reproducibility | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Low CD34 cells/µL | 12.15 | 252 | 2.15 | 17.68 | 0.00 | 0.00 | 0.15 | 1.26 | 0.00 | 0.00 | 0.15 | 1.26 |
| Low CD34% of CD45 | 0.18 | 252 | 0.03 | 18.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Low CD45 cells/µL | 6912.70 | 252 | 73.91 | 1.07 | 0.00 | 0.00 | 15.06 | 0.22 | 0.00 | 0.00 | 15.06 | 0.22 |
| Med CD34 cells/µL | 32.20 | 252 | 3.33 | 10.34 | 0.71 | 2.21 | 0.44 | 1.37 | 0.46 | 1.43 | 0.95 | 2.96 |
| Med CD34% of CD45 | 0.46 | 252 | 0.05 | 10.90 | 0.01 | 2.46 | 0.00 | 0.52 | 0.01 | 1.17 | 0.01 | 2.78 |
| Med CD45 cells/µL | 6922.56 | 252 | 56.08 | 0.81 | 29.45 | 0.43 | 16.77 | 0.24 | 7.80 | 0.11 | 34.78 | 0.50 |
| High CD34 cells/µL | 110.99 | 252 | 7.17 | 6.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.24 | 0.21 | 0.24 | 0.21 |
| High CD34% of CD45 | 1.61 | 252 | 0.10 | 6.41 | 0.01 | 0.41 | 0.00 | 0.00 | 0.01 | 0.47 | 0.01 | 0.62 |
| High CD45 cells/µL | 6907.84 | 252 | 63.51 | 0.92 | 8.18 | 0.12 | 7.45 | 0.11 | 2.43 | 0.04 | 11.33 | 0.16 |

**Table 6. Study 3 Results by Lot**

| Lot-to-Lot | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Mean Value | N | Repeatability | | Between- Days | | Between-Run | | Between-Lot | | Reproducibility | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Low CD34 cells/µL | 11.62 | 252 | 1.90 | 16.34 | 0.31 | 2.65 | 0.00 | 0.00 | 0.00 | 0.00 | 0.31 | 2.65 |
| Low CD34% of CD45 | 0.17 | 252 | 0.03 | 16.92 | 0.00 | 2.76 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.76 |
| Low CD45 cells/µL | 6959.24 | 252 | 95.34 | 1.37 | 22.08 | 0.32 | 0.00 | 0.00 | 5.57 | 0.08 | 22.77 | 0.33 |
| Med CD34 cells/µL | 34.09 | 252 | 3.45 | 10.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Med CD34% of CD45 | 0.50 | 252 | 0.05 | 10.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Med CD45 cells/µL | 6875.40 | 252 | 112.45 | 1.64 | 27.11 | 0.39 | 36.11 | 0.53 | 26.61 | 0.39 | 52.41 | 0.76 |
| High CD34 cells/µL | 114.52 | 252 | 6.60 | 5.77 | 0.77 | 0.68 | 1.43 | 1.25 | 1.57 | 1.37 | 2.26 | 1.97 |
| High CD34% of CD45 | 1.66 | 252 | 0.10 | 6.04 | 0.01 | 0.47 | 0.02 | 1.34 | 0.03 | 1.61 | 0.04 | 2.15 |
| High CD45 cells/µL | 6897.47 | 252 | 110.69 | 1.60 | 28.06 | 0.41 | 0.00 | 0.00 | 0.00 | 0.00 | 28.06 | 0.41 |

**Table 7. Study 3 Results by Operator**

| Operator to Operator | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Mean Value | N | Repeatability | | Between- Days | | Between-Run | | Between-Operator | | Reproducibility | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Low CD34 cells/µL | 11.93 | 252 | 1.93 | 16.20 | 0.63 | 5.25 | 0.09 | 0.74 | 0.00 | 0.00 | 0.63 | 5.30 |
| Low CD34% of CD45 | 0.17 | 252 | 0.03 | 16.28 | 0.01 | 5.61 | 0.00 | 1.97 | 0.00 | 0.00 | 0.01 | 5.95 |
| Low CD45 cells/µL | 6934.94 | 252 | 81.77 | 1.18 | 16.61 | 0.24 | 0.00 | 0.00 | 11.60 | 0.17 | 20.26 | 0.29 |
| Med CD34 cells/µL | 32.11 | 252 | 3.34 | 10.39 | 0.00 | 0.00 | 0.00 | 0.00 | 0.42 | 1.32 | 0.42 | 1.32 |
| Med CD34% of CD45 | 0.46 | 252 | 0.05 | 10.91 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 1.31 | 0.01 | 1.31 |
| Med CD45 cells/µL | 6911.14 | 252 | 70.35 | 1.02 | 0.00 | 0.00 | 12.93 | 0.19 | 0.00 | 0.00 | 12.93 | 0.19 |
| High CD34 cells/µL | 109.68 | 252 | 6.75 | 6.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| High CD34% of CD45 | 1.58 | 252 | 0.10 | 6.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| High CD45 cells/µL | 6926.21 | 252 | 65.69 | 0.95 | 28.50 | 0.41 | 16.11 | 0.23 | 1.24 | 0.02 | 32.76 | 0.47 |

- **Study 4**

A multi-site study was conducted at 3 sites using 3 levels of control material.

**Table 8. Range of control material**

| | | % Positive Cell | Expected Range | Absolute Number | Expected Range |
|---|---|---|---|---|---|
| Low | CD34 cells/μL | 0.19 | 0.12~0.26 | 13.7 | 9.7 ~ 17.7 |
| Med | CD34 cells/μL | 0.45 | 0.36~0.56 | 32.4 | 25.4 ~ 39.4 |
| High | CD34 cells/μL | 1.52 | 1.22~1.82 | 106 | 86.0~126.0 |

Three replicates on two separate runs per day for 5 days were assessed for between days, between runs, between sites, and total reproducibility %CV.

**Table 9. Study 4 Results**

| Sample | Mean | N | Repeatability | | Between-Days | | Between-Run | | Between-Site | | Reproducibility | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Low CD34 cells/ μL | 12 | 90 | 1.78 | 14.5 | 0.6 | 4.9 | 0.59 | 4.8 | 1.29 | 10.6 | 1.54 | 12.6 |
| Low CD34% of CD45 | 0.2 | 90 | 0.025 | 14.4 | 0.008 | 4.3 | 0.009 | 4.9 | 0.018 | 10.1 | 0.021 | 12.0 |
| Low CD45 cells/μL | 6942 | 90 | 143.82 | 2.1 | 66.78 | 0.8 | 0.000 | 0.0 | 0.000 | 0.0 | 66.8 | 0.8 |
| Med CD34 cells/ μL | 32 | 90 | 3.26 | 10.2 | 1.17 | 3.6 | 0.000 | 0.0 | 1.41 | 4.4 | 1.83 | 5.7 |
| Med CD34% of CD45 | 0.5 | 90 | 0.046 | 10.0 | 0.015 | 3.3 | 0.000 | 0.0 | 0.020 | 4.3 | 0.025 | 5.5 |
| Med CD45 cells/μL | 6960 | 90 | 122.0 | 1.8 | 9.50 | 0.1 | 0.000 | 0.0 | 24.20 | 0.3 | 25.99 | 0.4 |
| High CD34 cells/ μL | 108 | 90 | 6.390 | 5.9 | 0.000 | 0.0 | 2.948 | 2.7 | 6.862 | 6.3 | 7.469 | 6.9 |
| High CD34% of CD45 | 1.6 | 90 | 0.097 | 6.2 | 0.025 | 1.6 | 0.028 | 1.8 | 0.100 | 6.4 | 0.106 | 6.8 |
| High CD45 cells/μL | 6937 | 90 | 114.309 | 1.6 | 36.779 | 0.5 | 40.102 | 0.6 | 0.000 | 0.0 | 54.414 | 0.8 |

- **Study 5**

An Anticoagulant Interference Study was conducted and demonstrated that there were no statistically or clinically relevant differences between sample types/anticoagulants.

Single site testing at 3 sites was conducted using 6 levels of HPC-A ACD clinical samples. One lot of reagent and one operator/instrument per site tested 3 replicates per sample over 6 runs in 24 hours (sample stability limit).

Target concentrations of viable CD34 cells/µL:

- 17 CD34 cells/µL
- 35 CD34 cells/µL
- 75 CD34 cells/µL
- 100 CD34 cells/µL
- 500 CD34 cells/µL
- 1000 CD34 cells/µL

**Table 10. Study 5 Results, Site1**

| Target Concentration Viable CD34 cell/µL | Site 1 | | | | |
|---|---|---|---|---|---|
| | Parameters | Total CD34 cells/µL | Viable CD34 cells/µL | Viable CD45 cells/µL | Viable % CD34 of CD45 |
| 17 cells/µL | Mean | 16,96 | 16,14 | 3270,04 | 0,50% |
| | SD | 2,29 | 2,22 | 230,65 | 0,09% |
| | CV | 13,51% | 13,76% | 7,05% | 17,60% |
| 35 cells/µL | Mean | 37,03 | 36,78 | 6455,58 | 0,57% |
| | SD | 3,34 | 3,23 | 443,08 | 0,05% |
| | CV | 9,01% | 8,78% | 6,86% | 9,49% |
| 68 cells/µL | Mean | 68,74 | 68,42 | 4648,85 | 1,48% |
| | SD | 4,65 | 4,66 | 262,57 | 0,15% |
| | CV | 6,76% | 6,81% | 5,65% | 9,86% |
| 100 cells/µL | Mean | 92,90 | 92,69 | 5652,53 | 1,64% |
| | SD | 5,36 | 5,39 | 234,42 | 0,12% |
| | CV | 5,77% | 5,82% | 4,15% | 7,28% |
| 450 cells/µL | Mean | 471,55 | 469,43 | 28077,02 | 1,67% |
| | SD | 28,17 | 27,76 | 1762,08 | 0,08% |
| | CV | 5,97% | 5,91% | 6,28% | 4,95 % |
| 960 cells/µL | Mean | 904,27 | 893,36 | 75292,90 | 1,18% |
| | SD | 41,01 | 41,29 | 2274,17 | 0,06% |
| | CV | 4,54% | 4,62% | 3,02% | 4,93% |

**Table 11. Study 5 Results, Site2**

| Target Concentration Viable CD34 cells/µL | Site 2 | | | | |
|---|---|---|---|---|---|
| | Parameters | Total CD34 cells/µL | Viable CD34 cells/µL | Viable CD45 cells/µL | Viable %CD34 of CD45 |
| 17 cells/µL | Mean | 17,05 | 16,91 | 3364,35 | 0,51% |
| | SD | 2,09 | 2,05 | 271,88 | 0,06% |
| | CV | 12,23% | 12,15% | 8,08% | 12,14% |
| 35 cells/µL | Mean | 35,51 | 35,03 | 6494,72 | 0,54% |
| | SD | 4,15 | 3,96 | 198,25 | 0,05% |
| | CV | 11,68% | 11,29% | 3,05% | 10,85 % |
| 68 cells/µL | Mean | 68,53 | 68,41 | 4038,08 | 1,65% |
| | SD | 4,55 | 4,49 | 210,61 | 0,17% |
| | CV | 6,64% | 6,76% | 5,22% | 10,58 % |
| 100 cells/µL | Mean | 101,12 | 100,63 | 6650,66 | 1,52% |
| | SD | 5,95 | 5,92 | 296,61 | 0,10% |
| | CV | 5,89% | 5,88% | 4,46% | 6,79 % |
| 450 cells/µL | Mean | 442,64 | 440,05 | 29360,64 | 1,55% |
| | SD | 26,32 | 24,89 | 829,78 | 0,10% |
| | CV | 5,95% | 5,66% | 2,93% | 6,48 % |
| 960 cells/µL | Mean | 982,99 | 977,50 | 53124,13 | 1,84% |
| | SD | 44,27 | 43,31 | 1566,41 | 0,06% |
| | CV | 4,50% | 4,43% | 2,95% | 3,36 % |

**Table 12. Study 5 Results, Site3**

| Target Concentration Viable CD34 cells/µL | Site 3 | | | | |
|---|---|---|---|---|---|
| | Measuring parameters | Total CD34 cells/µL | Viable CD34 cells/µL | Viable CD45 cells/µL | Viable %CD34 of CD45 |
| 17 cells/µL | Mean | 17,07 | 16,76 | 3325,93 | 0,50% |
| | SD | 2,09 | 2,42 | 170,27 | 0,06% |
| | CV | 12,21% | 14,47% | 5,12% | 12,19% |
| 35 cells/µL | Mean | 32,73 | 32,05 | 19447,80 | 0,54% |
| | SD | 3,22 | 3,31 | 1393,72 | 0,06% |
| | CV | 9,85% | 10,32% | 7,17% | 10,55 % |
| 68 cells/µL | Mean | 74,89 | 73,41 | 25862,38 | 0,28% |
| | SD | 4,44 | 4,34 | 1400,05 | 0,01% |
| | CV | 5,92% | 5,91% | 5,41% | 5,00% |
| 100 cells/µL | Mean | 100,25 | 97,08 | 24993,61 | 0,39% |
| | SD | 5,64 | 5,48 | 1066,57 | 0,02% |
| | CV | 5,62% | 5,65% | 4,27% | 5,74% |
| 450 cells/µL | Mean | 447,49 | 422,49 | 106235,8 | 0,40% |
| | SD | 20,94 | 17,45 | 6060,55 | 0,03% |
| | CV | 4,68% | 4,13% | 5,70% | 7,21% |
| 960 cells/µL | Mean | 1005,11 | 1002,17 | 44158,79 | 2,27% |
| | SD | 24,14 | 23,76 | 2044,63 | 0,10% |
| | CV | 2,40% | 2,37% | 4,63% | 4,28% |

## Linearity

The ADAMII™ -CD34 Kit demonstrated linearity across the claimed ranges:
1-1000 CD34 cells/µL

## Interference

The effects of substance interference on ADAMII™-CD34 performance were evaluated following the CLSI EP7-A3 protocol. The substances in the table below have been tested and found to have no interference at the concentrations specified in the table.

| Substances | Concentration |
|---|---|
| Hemoglobin | 50 mg/dL |
| Gamma globulin | 1 % |
| Bilirubin | 10 mg/dL |
| Albumin | 7.5 mg/mL |
| G-CSF | 60 ng/mL |
| Intralipid | 250 mg/dL |
| Cyclophosphamide | 550 µg/mL |
| Doxorubicin | 0.25 µg/mL |
| Paclitaxel | 20 µg/mL |

## Sample Stability

Stored sample stabilities and stained sample stability were evaluated at the NanoEntek Research laboratory using mobilized peripheral blood (MPB), leukapheresis (HPC-A), fresh cord blood (FCB) and thawed frozen cord blood (TFCB) specimens. Samples have been stored at the following temperatures.

| Stability test | Sample type | Temperature |
|---|---|---|
| Stored | FCB | 20 to 25°C (Room temperature) |
| | MPB, HPC-A, TFCB | 2 to 8°C |
| Stained | MPB, HPC-A, FCB, TFCB | 2 to 8°C |

Based on the results of this study, we recommend starting sample preparation within 24 hours of collection for MPB and HPC-A and within 48 hours of collection for fresh cord blood. We recommend keeping stained samples on wet ice, and analyzing prepared samples within 1 hour of completion of RBC lysis. Frozen cord blood samples should be prepared immediately after thawing and analyzed immediately after completion of RBC lysis.

### Limitation

ADAMII™-CD34 Kit is designed for use on the ADAMII™ instrument. Refer to the ADAMII™ Instrument User Manual for more information.
Do not use the ADAMII™-CD34 Kit or slides beyond the expiration date.

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## Glossary of Symbols

| | |
|---|---|
|  | Caution, warning,<br>Consult accompanying documents |
| REF | Catalogue number/Reference number |
|  <br>www.nanoentek.com/eifu.php | Consult Instructions for Use<br>An electronic instructions for use (eIFU) indicator<br>(website address) may accompany the symbol when used to<br>indicate an instruction to consult an eIFU. |
| LOT | Lot number/Batch number |
|  | Use by<br>YYYY-MM-DD or YYYY-MM |
|  | Manufacturer |
| CE | CE marking |
| IVD | *In vitro* diagnostic medical device |
|  | Temperature limitation |
|  | Contains sufficient for <n> tests |
|  | Do not reuse |
|  | Do not use if package is damaged |
| Rx Only | For prescription use only<br>CAUTION: Federal (U.S.) law restricts this device<br>to saleby or on order of a physician. |
| US Corporation | US Corporation |
| European Corporation | European Corporation |
| EC REP | Authorized representative in the European Community |
| UK Representative | Authorized representative in United Kingdom |
| CH REP | Authorized representative in Switzerland |
| BRH | Authorized representative in Brazil |

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