



SCIENCE+INSIGHT

TECHNICAL BULLETIN

Plasma Methylation Sample Collection and Preparation Instructions

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BACKGROUND

Doctor's Data recently revised our plasma methylation kit to accommodate testing for adenosine. During method development and validation, the variability associated with sample handling had not been fully assessed to provide clear guidance as to the centrifugation conditions to yield plasma that was free of cellular sources of adenosine. Both leukocytes (white blood cells) and thrombocytes (platelets) remain active in their cellular metabolic processes after blood has been collected, and continue to release adenosine as energy is consumed by the living cells. It becomes essential to remove these cellular components from the harvested plasma in order to assure accurate determination of the plasma adenosine levels. We have observed several elevated results for adenosine that we suspect as being artefactual, or falsely elevated, as a result of inadequate centrifugation speed and time. The previous guidance to centrifuge at 3000 rpm for 10 minutes has been found to provide inadequate removal of the leukocytes and platelets in a whole blood sample.

REQUIRED ACTION

Whole blood collected into EDTA lavender-top tubes must be centrifuged within 15 minutes of collection. Blood must be centrifuged for 20 minutes at a centrifugation speed of 4000 rpm or higher to achieve a relative centrifugal force of 2000g *. Allow the centrifuge to stop without braking. Within 5 minutes of the centrifuge stopping, carefully remove the tube(s) from the centrifuge without re-suspending the buffy coat of blood cells that forms atop the red cell layer. Pipette the plasma from the top of the plasma layer to avoid pipetting from the plasma that contains cellular components. Transfer plasma in the specified volume to the labeled aliquot tubes. Do not freeze the plasma, as freezing may cause elevation of adenosine if cellular mitochondria that remain in suspension after centrifugation are ruptured upon a freeze-thaw cycle. Refrigeration provides adequate maintenance of stability of the measured components in the plasma methylation profile. Failure to adhere to these handling guidelines may result in falsely elevated adenosine levels.



Our collection instructions are being modified to reflect these changes.

* If you do not know the g-force or rpm of your centrifuge, it may not be suitable for processing plasma for accurate adenosine measurement. Centrifuge at the highest rpm setting for 20 minutes. The remainder of the methylation profile is valid.

Visit: <https://www.eppendorf.com/US-en/centrifuge-speed-calculator/> to estimate the g-force of your centrifuge. Call Doctor's Data Scientific Support at 800-323-2784 if you have questions or need assistance with these instructions.