# BloodLyz™

(*Kit for lysis of red blood cells in cytology specimens for cell-blocking*)
https://www.avbioinnovation.com/product/bloodlyz

**URL to download PDF of Safety Data Sheet (MSDS sheet):** http://alturl.com/icvww

10 tubes with 5 mL (10X) stock solution in each tube to be used after diluting to the 50 mL mark with 45 mL distilled water at room temperature to get working BloodLyz™ solution.
Ammonium Chloride Solution is recommended for the lysis of red blood cells (RBCs) in hemodiluted cytology specimens. It is buffered and optimized for gentle lysis of erythrocytes, with minimal effect on other nucleated cells. Because the reagent lacks any fixative agent, the cells remain fresh, unfixed after lysis of the contaminant red blood cells.

**Composition**:
$NH_4Cl$ and EDTA in water buffered with $NaHCO_3$ with a final pH of 7.2 - 7.6

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*Storage conditions*: 15–30°C. Keep away from light.
*Shipping conditions*: ambient
*Shelf life*: 12 months from date of manufacture

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**Introduction:**

*Problems and solutions related to blood contamination in cell-blocks:*

The cell-blocks prepared by centrifugation methods directly from blood contaminated specimens, the nucleated diagnostic cells are concentrated above the column of RBCs similar to the buffy coat (Figure 1a). This means that the diagnostic cells will not align along the bottom, which would be the cutting surface in the final FFPE of cell-block. This introduces unpredictability in the alignment of the diagnostic cells along the cutting surface of the final paraffin blocks (Figure 1b, 2). In addition, the paraffin sections of the blood-rich specimens interfere during processing for staining, especially immunostaining because of a floating and folding tendency of such sections (Figure 1,2,3).

The contaminant erythrocytes should be separated out or lysed for optimal cell-blocks. Alcohol-based and acid-based lysis with acetic acid would interfere with qualitative integrity, potentially affecting the results of ancillary tests such as IHC and molecular pathology tests [1].

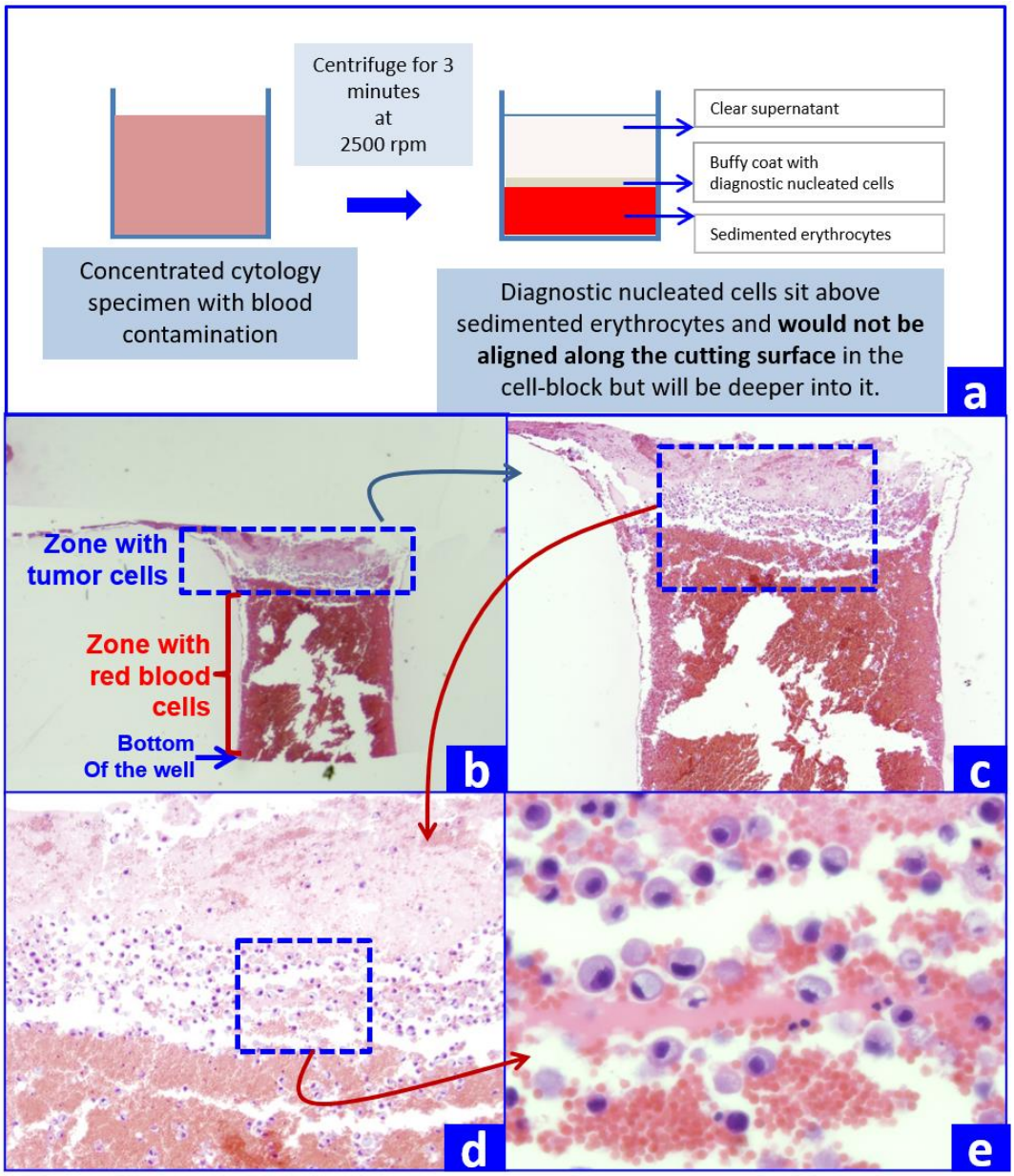


Figure 1. *Blood contaminated cytology specimen (H and E stain)*.
**a.** Centrifugation of the blood contaminated specimen with diagnostic cells which group with the nucleated cells in the buffy coat area above red blood cells.
**b.** A longitudinal section of one of the wells in *the cell-block made with Nano* NextGen CelBloking™ Kit.
**c.** The bottom of the well predominantly shows red blood cells and the diagnostic tumor cells on the top, which will be deep inside the actual cutting surface of usual cell-blocks.
**d** & **e.** Higher magnification showing the diagnostic tumor cells in the area corresponding with buffy coat.
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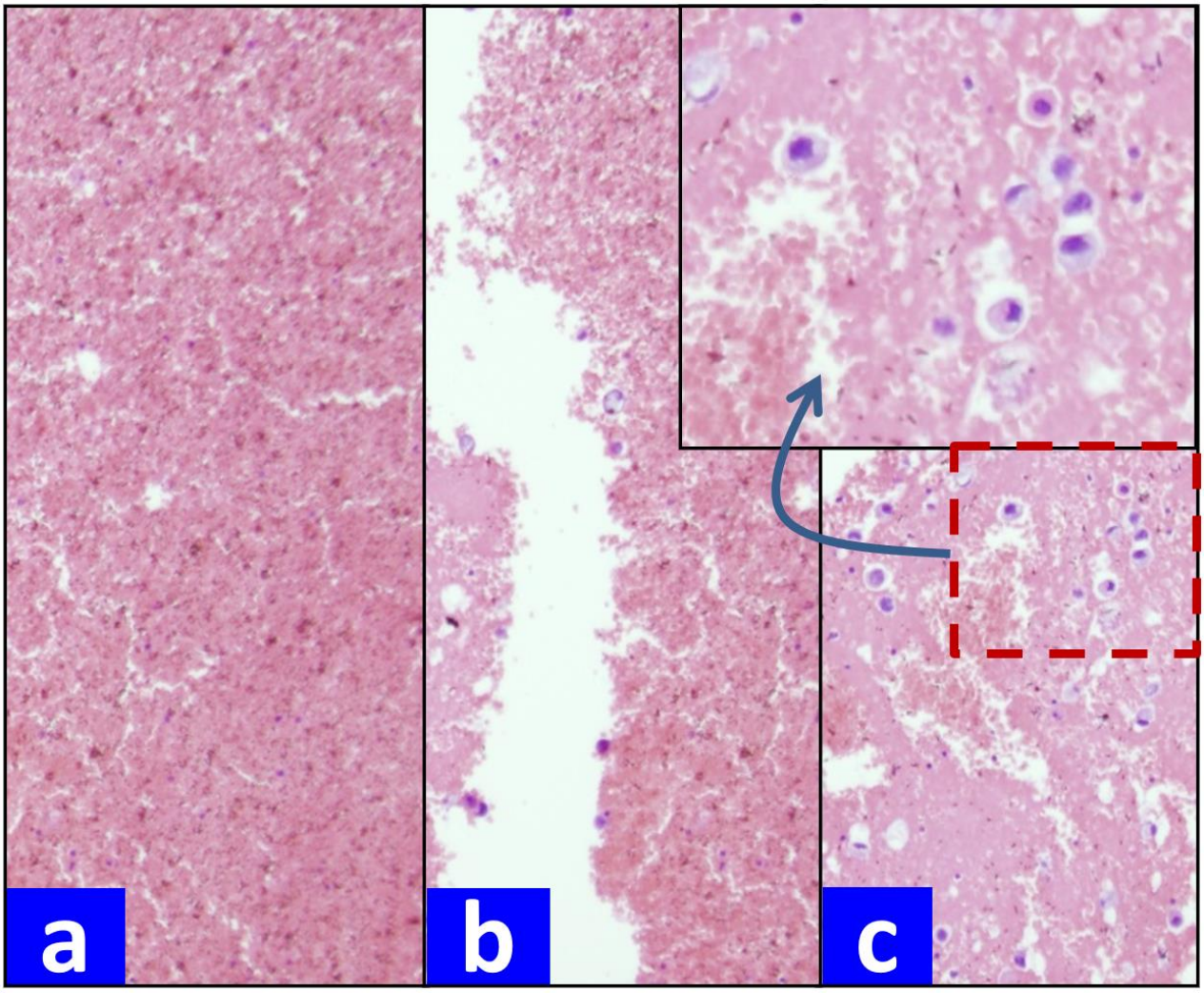


Figure 2. *Blood contaminated cytology specimen (H and E stain)*.
a & b: Blood interference in cell-blocks made using the Nano NextGen CelBloking™ Kit (same is applicable to any other method for blood rich specimens)
c: Section from the bottom of the wells with a specimen with sediments rich in red blood cells. The diagnostic tumor cell are missing in this zone.
The deeper levels showed more tumor cells, but this level cannot be predicted and so a possibility of catching the diagnostic cells depend on chance with frequent sampling artifact.
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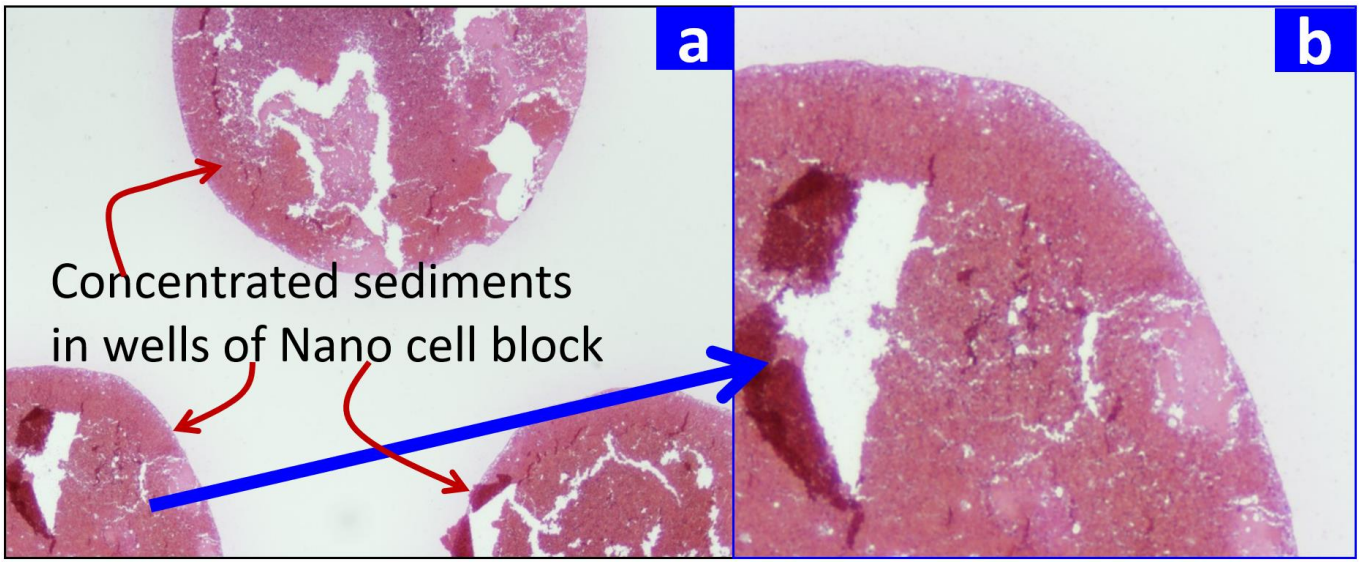


Figure 3. *Blood contaminated cytology specimen (H and E stain)*.
a. Low magnification of a section of cell-block made using the Nano NextGen CelBloking™ Kit. Theses sections have a tendency to float and fold, especially during immunostaining and other procedures requiring handling and processing through multiple reagents with problems related to floater contamination.
b. Higher magnification of the same section.
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Separation of diagnostic tumors cells and other nucleated cells may be achieved by gradient sedimentation methods comparable to separation of leucocytes as buffy coat by using media such as Ficoll-Hypaque. This method is expensive and needs significant skills and leads to non-reproducible results [2,3].
An ammonium chloride based erythrocyte lysing reagent such as BloodLyz™, similar to that used for flow cytometry is relatively simpler and inexpensive without compromising the immunoprofile integrity [3].

Specimens collected in different fixatives and collection/preservative media other than in isotonic medium or 10% formalin may interfere with results of immunostains and different molecular tests mostly standardized on FFPE.

Collecting directly in formalin will not allow for the making of cytology smear preparations. In addition, formalin collected specimens will not allow for the removal of blood contamination related interference.

Similarly in general, the practice of collecting cytology specimens in weak alcohol fixatives including Saccomanno Collection Fluid [4], various liquid based cytology collection media such as Cytolyt™, PreservCyt® (ThinPrep) [5], or CytoRich™ Red preservative (SurePath)

[6] would interfere with the IHC integrity and the results may not be comparable with the published data on FFPE based tissue.

Unfixed specimens, such as various body fluids, washings, and needle rinses in isotonic media such as Isotonic Medium S [7] allow for the lysis of erythrocytes and removal of blood contamination related interference [8].

For improved cell-blocks, such specimens should be depleted of RBC with a *lysing reagent* such as BloodLyz™ (ammonium chloride based lysing reagent similar to that used for flow cytometry), so that immunohistochemistry results are not affected.

The important preliminary step is the concentration of the cytology specimen to get most of the diagnostic sediment compacted in a small volume. This is usually achieved with the help of centrifugal force by centrifuging the specimen at 1250 RCF (2500 rpm on a centrifuge with a rotor diameter of 11 cm). The sediment button should be compact enough to avoid its dislodgement while decanting the supernatant by simple inversion of the tube into the discard container. 3 minutes of centrifugation is enough for a *specimen without blood* contamination; however, specimens with blood contamination usually have a relatively loose button and may require 5 minutes of centrifugation as a general guideline.

The final concentrated and RBC depleted nucleated diagnostic cells are cell-blocked by using the Nano NextGen CelBloking™ Kit [9] to achieve quantitative and qualitative (including immunointegrity comparable to FFPE) advantages along with the AV marker for objective guidance to histotechnologists while cutting the final FFPE to select the initial section with diagnostic material.

**Protocol for depletion of RBC interference in cell-blocking by lysing RBCs with BloodLyz™ (see Figure 4)**

1. For preparing working BloodLyz™ reagent, dilute 5 mL of concentrated 10X BloodLyz™ reagent provided in the tube by adding 45 mL of distilled water at room temperature up to the 50 mL mark.
2. Concentrate the specimen by centrifuging at 1250 RCF (2500 rpm on a centrifuge with rotor diameter of 11 cm) for 5 minutes. Decant supernatant by carefully removing the supernatant with a pipette.
3. Resuspend the sediments in the scant residual supernatant remaining after decanting.
4. Add working (diluted) BloodLyz™ to the blood contaminated concentrated sediment and disperse all the sediments in it (use up to 5 mL sediments for all 50 mL working BloodLyz™. If the sediments are less in quantity, you may economize and use a ratio of 0.5 mL sediment to 5 mL *working* (diluted) BloodLyz™. Extra unused working BloodLyz™ reagent may be stored at 2-8$^{o}$C and used within 7 days).

5. Cap securely and mix well by inverting gently a few times.
6. Wait for 5 minutes (not more than 10 minutes) at room temperature.
7. Centrifuge immediately at 2500 rpm for 3 minutes at room temperature.
8. Decant the reddish transparent hemolyzed supernatant.
9. Resuspend the nucleated cells in the whitish sediments and procced with cell-blocking protocol (without significant delay) using the Nano NextGen CelBloking™ (NGCB) Kit by adding the RBC-depleted concentrated sediment to the Nano NGCB unit [9].

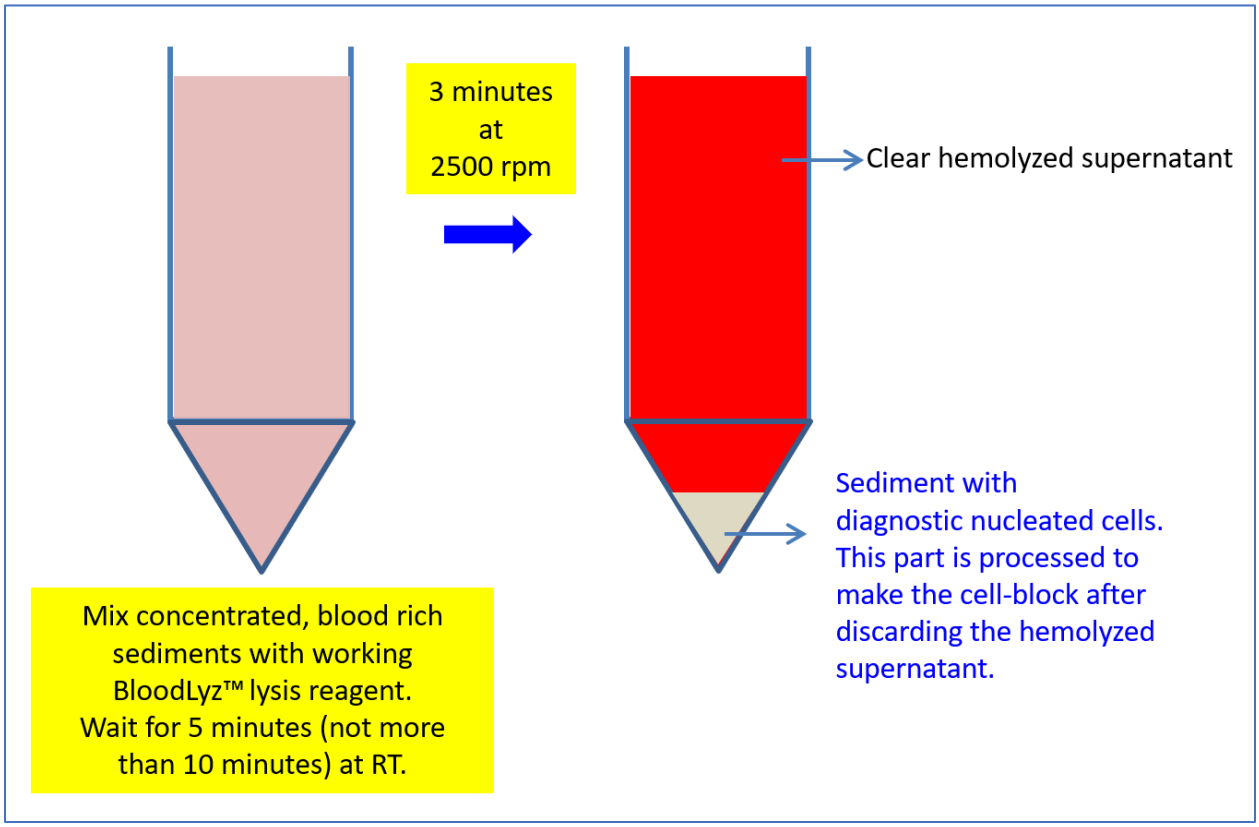


Figure 4. Processing of blood contaminated cytology specimens with BloodLyz™ to nullify the problems related to red blood cell contamination.
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3. Muirhead KA, Wallace PK, Schmitt TC, Frescatore RL, Franco JA, Horan PK Methodological considerations for implementation of lymphocyte subset analysis in a clinical reference laboratory. Ann N Y Acad Sci. 1986;468:113-127.

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https://www.bd.com/en-us/offerings/capabilities/cervical-cancer-screening/non-gyn-cytology/cytorich-fixatives

7. AV BioInnovation, Isotonic Medium S™
https://www.avbioinnovation.com/product/isotonic-medium-s

8. Shidham VB. *CellBlockistry*: Chemistry and art of cell-block making- A detailed review of various historical options with recent advances (Review).
*CytoJournal* 2019, 16:12 (28 June 2019)
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http://www.cytojournal.com/temp/CytoJournal16112-3882578_104705.pdf
DOI:10.4103/cytojournal.cytojournal_20_19

9. Processing of a single specimen of any cellularity to make a cell block with Nano Unit.
https://youtu.be/y29SS1NwO_8

For additional questions, please contact Technical Support at Phone: 262 797 0323
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