



## NextGen CelBloking™ Kit

N20; M20; C20: For making 20 cell-blocks

T2: Trial pack for making 2 cell-blocks

*For in vitro use only*

[www.AVBioInnovation.com](http://www.AVBioInnovation.com)

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Patent Pending

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N20; M20; C20: For making 20 cell-blocks  
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**Cell-block making kit with built-in AV marker for  
Quantitatively and Qualitatively enhanced cell-blocks.**



[https://youtu.be/y29SS1NwO\\_8](https://youtu.be/y29SS1NwO_8)

Watch **Nano** structure & function with  
procedure for one specimen



<https://youtu.be/i-ZpXaljils>

Watch **Micro** structure & function with  
procedure for one cellular specimen

### **Introduction:**

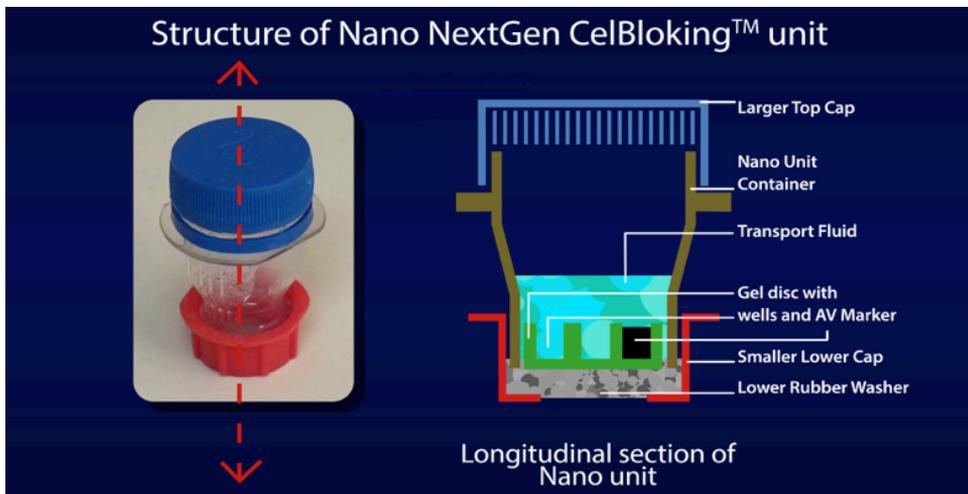
Cell-blocks are an important part of the cytopathologic evaluation process of various cytology/tissue specimens. However, routine methods have many limitations with relatively frequent suboptimum outcomes due to randomness in various steps of the process, compromising the desired outcome.

Routine random methodologies may compromise the qualitative integrity of the specimen due to exposure to fixatives and reagents during the cell-block-making protocol. This may not produce the final outcome of ancillary studies such as immunohistochemistry and molecular tests comparable to the results on Formalin-Fixed Paraffin-Embedded (FFPE) tissue of surgical pathology biopsies, potentially undermining the management of the disease and its final outcome.

Many specimens have diagnostic cells in it, but conventional methodologies do not have any control over their retrieval in tissue sections under study due to the randomness of the location of diagnostic cells in the final cell block. NextGen CelBlok™ Kits are based on Shidham's method (1) in which the diagnostic cells in the sediments of the specimen are concentrated and aligned along the cutting side of the final cell-block.

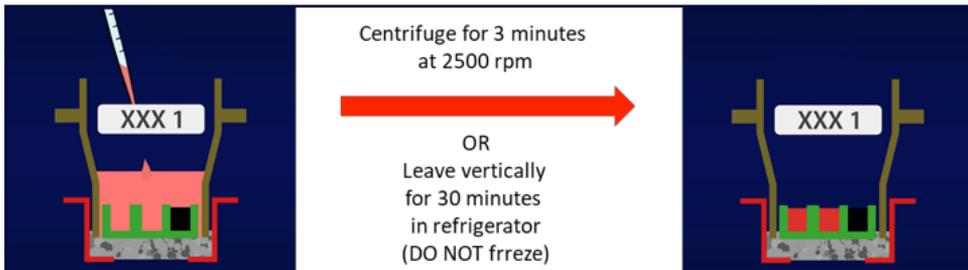
Similarly, the location of the cells in the cell-block may not be easily assessed by the histotechnologist cutting the block. This leads to a lack of control over the depth at which to select the sections for studying. In some cases, the histotechnologist may not have even reached the depth with diagnostic cells, resulting in the absence of any diagnostic cells in the sections. In other cases, the histotechnologist may cut through the scant diagnostic cells and lose them all. NextGen CelBlok™ Kits have pre-formed disc medium with wells and precisely aligned built-in AV markers. The dark-colored AV marker allows visual confirmation by the histotechnologist for objective confirmation of the depth at which the diagnostic cells (aligned along the bottom of the wells in the preformed medium disc) appear in the sections. This overcomes the randomness of conventional methods and allows precision in controlling the alignment of the diagnostic cells in initial sections of the cell blocks even in relatively hypocellular specimens.

## Structure of Nano unit:



## Function of Nano unit:

See video [https://youtu.be/y29SS1NwO\\_8](https://youtu.be/y29SS1NwO_8)



## Procedure for Nano units:

### a. Procedure for a single specimen:

Please watch the video "Preparation of cell-block from specimens of any cellularity with Nano NextGen CelBloking™ unit" at: [https://youtu.be/y29SS1NwO\\_8](https://youtu.be/y29SS1NwO_8)

The summary of procedure for single specimen is outlined below:

## Summary of Procedure for Single Specimen



Label the Nano Unit



Discard the transport fluid in Nano Unit



Add concentrated specimen to Nano Unit



Centrifuge at 2500 rpm for 3 minutes and discard the supernatant



Add 10% formalin



Centrifuge at 2500 rpm for 3 minutes and discard the supernatant



Dislodge the gel disc into tissue cassette



Cover gel disc with Tissue paper cover



Close the tissue cassette and put in horizontal position in transport container with 10% formalin. Transfer the container for tissue processing.

NanoX1

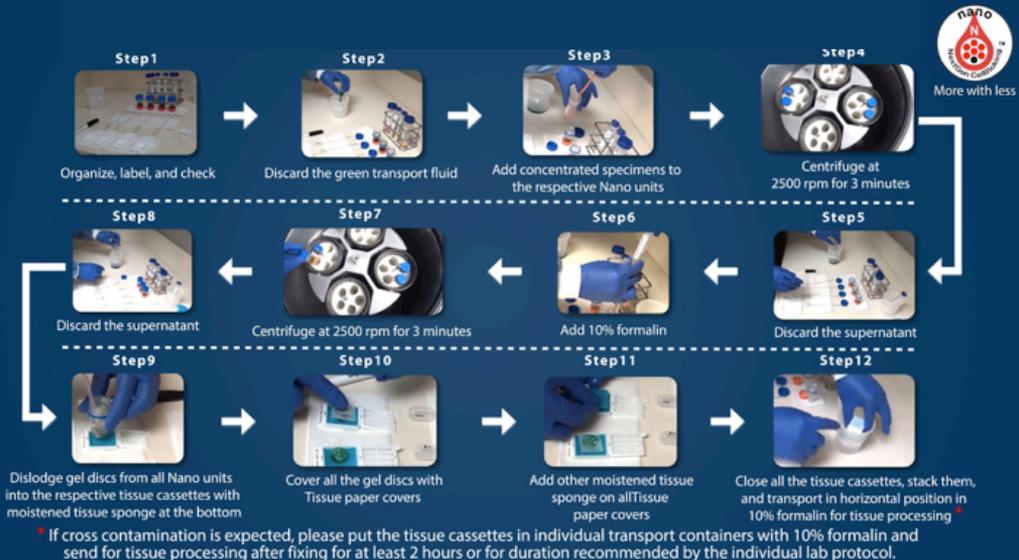


**b. Procedure for multiple specimens simultaneously:**

Please watch the video “Processing multiple specimens of any cellularity for cell-block making with Nano NextGen CelBloking™ unit” at: <https://youtu.be/ZPb0nq8MsLk>

The summary of procedure for multiple specimens is outlined below:

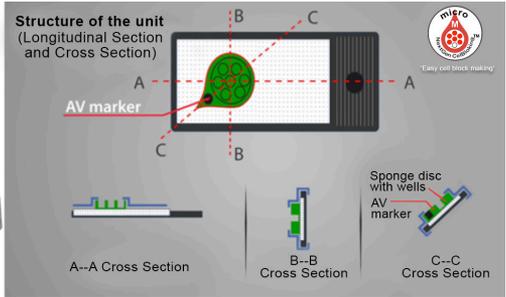
## Summary of Procedure for Multiple Specimens



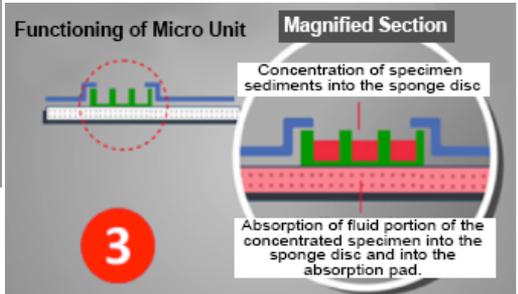
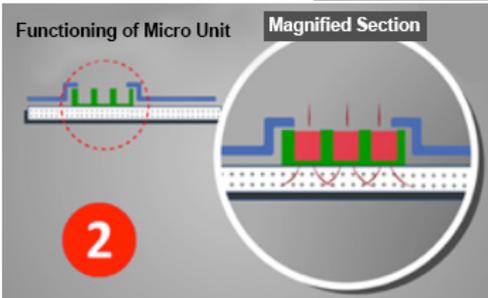
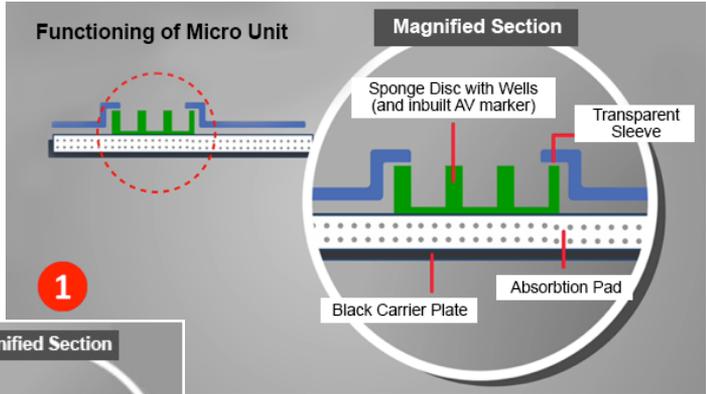
**NanoX4**



Structure of Micro unit:



See video  
<https://youtu.be/i-ZpXajils>



## Procedure for Micro units:

### a. Procedure for a single specimen:

Please watch the video "Preparation of single **sediment rich specimen** to make a cell-block with

**Micro NextGen CelBloking™** units" at: <https://youtu.be/i-ZpXajjils>

The summary of procedure for single specimen is outlined below:

## Summary of Procedure for Single Specimen

### STEP - 1



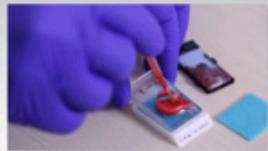
Add concentrated specimen on the sponge disc.

### STEP - 2



Let the fluid portion of the specimen settle down into the absorbent pad for ten minutes. After addition of formalin fixative, allow to diffuse into the absorbent pad for another ten minutes.

### STEP - 3



Transfer the sponge disc with concentrated specimen sediments into the tissue cassette.

### STEP - 5



Let the cassette fix in horizontal position with bottom down for more than 2 hours.

### STEP - 4



Put the sponge disc with concentrated sediments and covered with tissue paper cover between two tissue sponges in tissue cassette.

MicroX1



### b. Procedure for multiple specimens simultaneously:

Please watch the video “Simultaneous processing of multiple concentrated cellular specimens with Micro NextGen CelBloking™ units” at: <https://youtu.be/TRW5Vswy6J8>

The summary of procedure for multiple specimens is outlined below:

## Summary of Procedure for Multiple Specimens

STEP - 1



Add concentrated specimens on the respective sponge discs and keep the respective transfer pipettes in their places for use at later stage to dislodge the respective sponge discs with concentrated specimen into the respective tissue cassettes.

STEP - 2



Add 10% formalin fixative to all sponge discs with concentrated specimen.

STEP - 5



Let all the cassette fix in horizontal position with bottom down for more than 2 hours. If cross-contamination is anticipated, fix and transport each tissue cassette individually (instead of stacking and transporting multiple tissue cassettes in one container shown in this demo).

STEP - 4



Put all the sponge discs with concentrated sediments and covered with tissue paper cover between two tissue sponges into their respective tissue cassettes.

STEP - 3



Transfer the sponge discs with the tip of the transfer pipette into their tissue cassette

### MicroX5



### Tip for best results:

Process the original specimen to get maximum volume of concentrated specimen with highest possible Tissucrit.

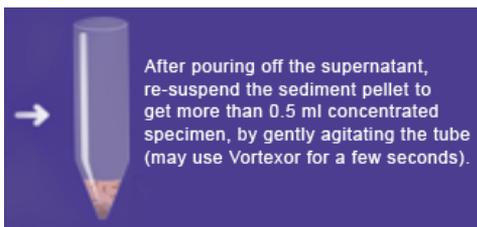
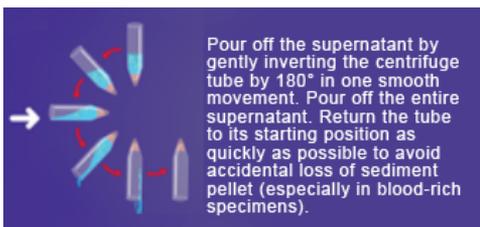
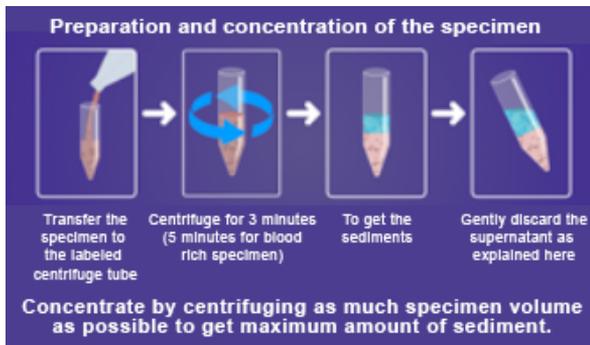
### Tissucrit (% sediment)



Tissucrit  
(=proportion of sediment in percent)

Guesstimated proportion of sediment to the supernatant of final concentrated specimen

*Specimen processing to get concentrated specimen:*



*Specimen collection:*

The specimens processed for cell-block making should be unfixed (fresh) to start with. Therefore, specimens such as fine needle aspiration (FNA) biopsy needle rinses and dedicated passes for cell-blocks should be collected in an isotonic medium (e.g., RPMI, saline, IsotonicMediumS™ [www.AVBioInnovation.com](http://www.AVBioInnovation.com)). In the event that the specimen must be collected in fixative, then it should be directly collected in 10% formalin. It should not be collected in any other fixatives/collection media such as Saccomanno, CytoLyt etc. Use of other reagents/fixatives may lead to aberrations in results on ancillary studies such as immunohistochemistry, molecular pathology, etc., which are usually standardized on formalin fixed paraffin embedded tissue.

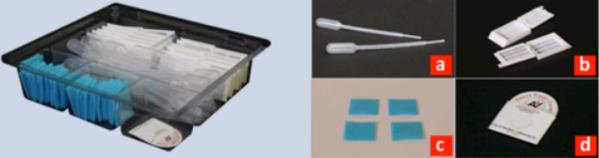
**Trouble shooting:**

For specimens with a high proportion of **blood contamination**, first lyse the blood (using BloodLyz™ reagent kit [www.AVBioInnovation.com](http://www.AVBioInnovation.com)) and then proceed with cell-block making from the concentrated specimen with predominance of diagnostic component.

If a centrifuge with a free swinging rotor for 50 ml tubes is not available, the sedimentation steps used in the procedure twice could be replaced by **natural gravity**. For the steps otherwise needing centrifugation, leave the Nano units undisturbed for 30 minutes in cold (such as in a refrigerator, DO NOT allow to freeze). Then discard the supernatant gently with the help of a transfer pipette. Note that simply inverting the Nano unit to discard the supernatant may not be safe because the sediments by gravity may not be compact as achieved by centrifugation.

### Kit content and storage:

All NextGen CelBloking™ Kits are **supplied as 2 packs** (Pack #1 with units and Pack #2 with important ancillary supplies).

	Nano Catalog #N20	Micro Catalog #M20	Combo Catalog #C20
<b>Pack #1</b>			
	<p>20 <b>Nano</b>- NextGen CelBloking™ units, each unit contains            One preformed gel disc with wells and precisely set built-in black AV marker.            Up to 2 ml transport fluid aqueous, with edible green color with traces of formalin as preservative.  <b>Store below 45° F (8° C) - Do NOT freeze</b></p>	<p>20 <b>Micro</b>- NextGen CelBloking™ units, each unit contains            One preformed foam disc with wells and precisely set built-in black AV marker.</p> <p><b>Store in a dry place at room temperature</b></p>	<p>Combo pack with            10 <b>Nano</b>- NextGen CelBloking™ units,            AND            10 <b>Micro</b>- NextGen CelBloking™ units,</p> <p>(For other details please see Nano and Micro)  <b>Store below 45° F (8° C) - Do NOT freeze</b></p>
<b>Pack #2</b>	<p>a. 20 Transfer pipettes            b. 20 Tissue cassettes            c. 40 Tissue sponges            d. 1 envelope pack with 20+ tissue paper covers</p> <p>See video on "How to Remove tissue paper covers from its envelope pack"  <a href="https://youtu.be/oZSY0-iAy7k">https://youtu.be/oZSY0-iAy7k</a></p> <p style="text-align: center;"><b>Store in a dry place at room temperature</b></p> <div style="display: flex; align-items: center;">   </div>		

 <p><a href="https://youtu.be/1OfzS1dC28w">https://youtu.be/1OfzS1dC28w</a></p>	 <p><a href="https://youtu.be/hlsgHRl6J5l">https://youtu.be/hlsgHRl6J5l</a></p>
<p>Nano unit: Depiction of open arrows on large upper cap And Open reverse (anticlockwise) arrows for small lower cap</p>	<p>How to twist open the seal of upper large cap of Nano unit with hand</p>
 <p><a href="https://youtu.be/RlMN8lXh4s0">https://youtu.be/RlMN8lXh4s0</a></p>	 <p><a href="https://youtu.be/h2yuWg8H4pM">https://youtu.be/h2yuWg8H4pM</a></p>
<p>How to open the seal of upper large cap of Nano unit with blunt scalpel or knife (if it could not be twist broken with hand)</p>	<p>Nano &amp; Micro cell-block discs after tissue processing Instructions to histotechnologists</p>

Videos below contain details on the following:

### Safety information:

Wear appropriate skin and eye protection throughout the procedure with universal precautions for biological specimens to be considered potentially infectious. Safety data sheets (SDS) are available

Kit component	GHS	Hazard Phrases	Precaution phrases
<p>Preformed gel discs with Green transport fluid in Nano units contain very dilute formaldehyde (less than 0.074% in 1.5 ml per unit)</p>		<p>H302 Harmful if swallowed. H317 May cause an allergic skin reaction. H318 Causes serious eye damage. H319 Causes serious eye irritation. H351 Suspected of causing cancer.</p>	<p>P303+P361+P353 IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a POISON CENTER/doctor. P501 Dispose of contents/container in accordance with local/regional/national/ international regulations.</p>

at our “Resource Center” on our webpage [www.AVBioInnovation.com](http://www.AVBioInnovation.com)

### References:

1. Varsegi G.M., Shidham V. (2009). Cell-block Preparation from Cytology Specimen with Predominance of Individually Scattered Cells.

J Vis Exp. (JoVE- Journal of Visualized Experiments) 2009 Jul 21;(29). pii: 1316.

JoVE. 29.

doi: 10.3791/1316. PMID: 19623160

Video article is available FREE on web as open access at-<http://www.jove.com/index/Details.stp?ID=1316>



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