

OSUCCC Leukemia Tissue Bank Protocol: Buccal Swab DNA Isolation with DNeasy Kit

OSUCCC LTB Laboratories Procedure Buccal Swab DNA Isolation with DNeasy Kit ¹		Effective: 10/01/1997	
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Procedure No.	Supersedes:	Created/Review Date:	Number of Pages:
Version 1.0:		10/01/1997	2
		9/30/1998	2
		9/30/1999	2
		9/30/2000	2
		9/30/2001	2
		9/30/2002	2
Version 1.1 (Angela Lang, Lab Services Coord.)	V1.0	8/22/2003	3
		9/30/2003	3
		9/30/2004	3
		9/30/2005	3
		9/30/2006	3
Version 1.2 (Isabel Manring, Clinical Lab. Technologist)	V1.1	4/12/2007	3
		9/30/2007	3
		9/30/2008	3
		9/30/2009	3
		8/11/2010	3
		9/17/2011	3
		9/21/2012	3
Version 1.3 (Wacharaphon Vongchucherd, Clinical Lab Technologist)	V1.2	9/20/2013	3
		11/17/2014	3
		11/30/2015	3

1.0 PRINCIPAL

The DNeasy Tissue system uses advanced silica-gel-membrane technology for rapid and efficient isolation of total cellular DNA without phenol, chloroform, or ethanol precipitation. Mechanical homogenization is not necessary since tissues are directly lysed by proteinase K, reducing hands-on time. The buffer system is optimized to allow selective binding of DNA to the DNeasy membrane. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins, divalent cations, and secondary metabolites.¹

2.0 SPECIMENS

¹ T:\HCG\Caligiuri lab\Procurement\Lab Manual\Protocols\ALLIANCE-OSU LTB

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Buccal swabs are collected as specified by ALLIANCE protocol 9665; The Leukemia Tissue Bank². The protocol specifies collection of buccal swabs at pretreatment, remission and relapse time periods, although only pretreatment buccal swab collection is mandatory for all patients enrolled on ALLIANCE 9665.

3.0 MEDIA AND SUPPLIES

DNeasy kit components

- ATL Buffer,
- Proteinase K,
- RNase A,
- AL Buffer,
- AE Buffer
- AW1 Buffer,
- AW2 Buffer,

100% Molecular Grade Ethanol

Micropipetter (1000µl and 200µl)

Sterile pipet tips (1000µl and 200µl)

Scissors and forceps

4.0 EQUIPMENT

Incubator/Shaker

1.5 mL Microcentrifuge

Vortex mixer

Microcentrifuge tubes

Pipetters

5.0 QUALITY CONTROL AND SAFETY

All samples should be handled using universal precautions for biohazards. It is recommended that specimen collection be carried out in accordance with NCCLS document M29T2. No known test sample can offer complete assurance that human blood samples will not transmit infection. Therefore, all derivatives are potentially infectious. Ethanol is flammable.

6.0 PROCEDURE

1. Place the brush of the buccal swab into a 1.5 mL microcentrifuge tube and add 180 µL Buffer ATL.
2. Cut off the head of the buccal swab while it is in the 1.5 mL microcentrifuge tube.
3. Add 20 µL Proteinase K and mix by vortexing. Incubate at 55° C and shake at 1000 rpm until the tissue is completely lysed (approximately 3-4 hours). A low 260/280 ratio can be a result of inadequate lysis.
4. (Optional) Add 5.5 µL of RNaseA, mix by vortexing, and incubate for 2 minutes at room temperature.
5. Vortex for 15 seconds. Add 200µl Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70° C for 10 minutes.
6. Add 200µl ethanol to the sample, and mix thoroughly by vortexing.
7. A white precipitate may form on addition of ethanol. It is important to apply all of the precipitate to the DNeasy Mini spin column.

² T:\HCG\Caligiuri lab\Procurement\Lab Manual\Protocols\ALLIANCE-OSU LTB ALLIANCE – OSU LTB Laboratories Procedure Collection of Buccal swab for PCR analysis

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8. Pipette the mixture from step 5 into the DNeasy Mini spin column placed in a 2 mL collection tube. Centrifuge at 8000 rpm for 1 minute. Discard flow-through and collection tube.
9. Place the spin column in a new 2 mL collection tube, add 500 μ L buffer AW1, and centrifuge for 1 minute at 8000 rpm. Discard the flow-through and collection tube.
10. Place the spin column in a 2 mL collection tube (provided).
11. Add 500 μ L Buffer AW2, and centrifuge for 3 minutes at 14000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.
12. Be sure that the spin column does not touch the flow-through since this will carry over ethanol residue. If this happens, empty out the collection tube and reuse it for another centrifugation for 1 minute at 14000 rpm.
13. Place the spin column in a clean 1.5 mL microcentrifuge tube, and pipet 25 μ L Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 minute, and then centrifuge for 1 min at 8000 rpm to elute.
14. Repeat elution once as described in step 8 using 25 μ L Buffer AE.
15. Prepare a blank using AE Buffer.
16. Prepare samples for evaluation by Nano-Drop using 1.5 μ L of each sample.

7.0 LIMITATIONS

All buffers AW1 and AW2 must be prepared according to the directions on the bottles and the DNeasy Tissue Handbook. If precipitate forms in the AL and ATL buffer, incubate the buffers at 55° C until the precipitate has fully dissolved. Prepare a 55° C incubator/shaker for use in step 2 and a 70° C incubator/shaker for use in step 3. Preparing any more than 20 DNA samples at one time is not advisable.

8.0 REFERENCES

1. DNA EZ Tissue Handbook. Qiagen Corporation. 2005.
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