

NRG-BR002 -1/27/16

Optional Study #1: Blood Collection for Translational Research: Circulating Tumor Cells (CTCs)

Kit Contents: CTC kits are supplied with the Banking Kits (optional Study#3) from the NRG Oncology Biospecimen Bank- San Francisco and include enough supplies for the 2 Timepoints: Pre-tx and Post-Tx:

- Two CTC tubes
- Two Styrofoam tube holders with two outer cardboard containers
- Two Empty Fed Ex pouch
- Instructions for packing and shipping
- ST Form for the CTC shipment with Dr Woodward's address

Instructions:

Consenting patients will have 7.5 ml of whole blood drawn by phlebotomy or through a central venous access device into a "CellSave" tube to collect and quantify CTCs. Because the analytes for this study degrade rapidly they must be only collected Monday-Wednesday and shipped overnight

- 1) Samples for CTC analysis must be drawn on a Monday, Tuesday or a Wednesday in "CellSave" blood collection tubes containing EDTA and a stabilizer (Immunicon Corporation).
- 2) CTC tube must be clearly labeled with patient study number, type of sample, and date collected.
- 3) The tube should be inverted to mix then placed into the provided Styrofoam container.
- 4) The Styrofoam container should be placed into the fitted cardboard container, closed and placed into a plastic biohazard bag and outer paper envelope.
- 5) The sample and one CTC- special completed ST form then gets placed into a Fed Ex overnight Clinical Pak envelope and the envelope is sealed.
- 6) Place the label on the outside of the Clinical Pak- See below for label information.
- 7) Shipping days: Mon-Wed. ONLY (U.S. sites); Mon-Tues. (Canada and Non-North American). And NO later than Thursday to MD Anderson Cancer Center will be accepted.
- 8) For Fed Ex label go to: www.Fedex.com or use a paper handbill.
 - a. Bill third party FEDX account number 112964665 –ONLY to be used for this purpose.
 - b. **Always** include "NRG-BR002CHMURA" in the first reference field.
 - c. If using FedEx online include "BR002CTC-Case#" in the second ref field
 - d. If using FedEx online include your email and Dr Carol Hall and Dr Woodward's email in the email notifications
 - e. Ship Priority Overnight (by 10.30 am). There is **NO** Saturday delivery permitted.
 - f. Ship "CTC" tube materials (**labeled NRG-BR002**) directly to:

Carol Hall
UT MD Anderson Cancer Center
Dept of Surgical Oncology
1515 Holcombe Blvd
Tan Zone, room T4.3932
Houston , TX 77030
713-563-8898/FAX 713-794-4830

- 9) **MANDATORY:** Notify both Dr. Carol Hall (cshall@mdanderson.org) and Dr. Wendy Woodward (wwoodward@mdanderson.org) by e-mail on the day of submission with the following information:
 - that a sample is being submitted with the NRG case number;
 - the overnight shipping carrier and tracking number, and
 - e-mail and phone number of contact person.



NRG Oncology

Specimen Transmittal Form
Special Cell Save Blood Tubes

Study# NRG-BR002

Case #

PLACE LABEL HERE

Institution

Institution No.

Participant's Initials

Participant's I.D. No.

INSTRUCTIONS: This form must be completed and mailed with the specimens upon submission. Please see protocol for list of required materials. Use one separate form for each timepoint.

STATUS	SPECIMEN OBTAINED DATE	TIME POINTS (per section 10 of the protocol)	*SPECIMEN TYPE (see table below)	NUMBER OF SPECIMENS	STORAGE METHOD	PATHOLOGY ACCESSION# / PT ID#
1	____ - ____ - ____ MM DD YYYY	T-0	99 - Blood for CTC			
4	____ - ____ - ____ MM DD YYYY	Arm-1 3 months	99 - Blood for CTC			
4	____ - ____ - ____ MM DD YYYY	Arm-2 4 weeks	99 - Blood for CTC			
	____ - ____ - ____ MM DD YYYY					
	____ - ____ - ____ MM DD YYYY					
	____ - ____ - ____ MM DD YYYY					

----- STATUS -----			
1	Pre-treatment	6	Recurrence
2	Surgical treatment	7	Autopsy
3	During treatment	99	Unknown
4	Post-treatment		

ENCLOSURES:

_____ Specimens - Blood for CTC

_____ This Submission Form

SEND TO:

FedEx/Courier address

Dr. Carol Hall
UT MD Anderson Cancer Center
Dept. of Surgical Oncology
1515 Holcombe Blvd.
CRB T4.3932
Houston
Texas 77030
713-563-8898
Fax: 713-794-4830
wwoodward@mdanderson.org

***SPECIMEN TYPE**

- 10 H & E Stained Slides (P2)
- 11 Paraffin Blocks (JB)
- 12 Punch Bx (JH)
- 13 Unstained Slides (JN)
- 14 Fresh Tissue (JF)
- 30 Whole Blood (JW)
- 31 Serum (JS)
- 32 Plasma (JP)
- 33 Buffy Coat (JK)
- 50 Urine (JU)
- 51 Saliva (JL)
- 52 Buccal Scrapings (JR)
- 60 CSF (JC)
- 99 Other, specify (JT)

Check all that apply.

Patient consents to:

- 1 Current research as specified in the protocol
- 2 Cancer research
- 3 Medical research
- 4 Being contacted about future research

SUBMITTED BY: _____

TELEPHONE NO: (_____) _____ EMAIL: _____

NRG-BR002: 2/8/2016

Optional Study #2: Plasma Collection for Translational Research: ctDNA analysis

Please note that we have only included the BR002 Streck Tube Kit for the pre-treatment timepoint for this special study.

Once you have enrolled and used this kit please contact us and request the second timepoint kit. Allow 5-7 business days for delivery.

All un-used Streck tube kits must be shipped back to the NRG-SF within 6 months inside the provided outer brown cardboard box. They may not be discarded. Please keep the kit outer cardboard box until you use the kit.

Questions?: email us at NRG@UCSF.edu or call us at 415-476-7864

Streck Kit Contents: 1 Timepoint

- One (1) outer cardboard box
- One (1) Special shipping Box with Special Air Pillow Bag
- One (1) Special shipping Cassette
- One (1) temperature monitoring pouch
- One (1) streck tube in plastic holders
- Orange Temperature Monitor Instructions
- Kit Instructions (this piece of paper)
- Instructions for packing and shipping to Dr Kuhn
- ST Form with Dr Kuhn's lab instructions/address

Continued on Back page:

Streck Kit & Shipping Instructions:

- 1) A special plasma tube manufactured by Streck (7-10 mL) will be drawn at the same time points as the CTC collection into a “Streck” tube for eventual ctDNA cell-free analysis, and should be shipped to The Kuhn Laboratory at USC (see address below).
- 2) **NOTE:** Guidelines for the shipment of ctDNA/“Streck” tube:
The Biospecimen Bank kit will include special shipping container for returning the Streck tube. This includes a white, two part “cassette” to hold the blood tube, with monitoring pouch that goes inside the cassette an air pillow bag, and a shipping box.- See separate instructions about how to pack and ship this samples.
- 3) Include one Streck tube specific ST form per timepoint with shipment and One Orange tracking sheet
- 4) Shipping days: Mon-Wed.(U.S. sites); Mon.-Tues. (Canada and Non-North American).
- 5) For Fed Ex label go to: www.Fedex.com or use a paper handbill.
 - i. Bill third party FEDX account number 112964665 –ONLY to be used for this purpose.
 - ii. **Always** include “**NRG-BR002CHMURA**” in the first reference field.
 - iii. If using FedEx online include “Streck-Case#” in the second ref field
 - iv. If using FedEx online include your email and Dr Kuhns lab email in the email notifications
 - v. Ship Priority Overnight (by 10.30 am). **Saturday deliveries:** see attached shipping instructions from Kuhn Lab
 - vi. Ship “Streck” tube ctDNA materials (**labeled NRG-BR002**) directly to:

Samples collected Mondays-Thursdays ship to:
USC, Kuhn Laboratory
3430 S. Vermont, TRF 125
Los Angeles, CA 90089
Phone: 213-821-3051
kuhnlab@usc.edu

Samples collected Fridays ship to:
USC, Kuhn Laboratory
Attn: (name of on-call person)
840 Childs Way
Los Angeles, CA 90089
Phone: 213-746-4234

- 6) Notify Dr. Wendy Woodward (wwoodward@mdanderson.org) and the Kuhn lab (kuhnlab@usc.edu) by e-mail on the day of submission with the following information:
 - That a sample is being submitted with the NRG case number;
 - Shipping carrier and tracking number
 - E-mail and phone number of contact person.

For questions call Mariam Rodriguez Lee at 858-405-9347 (mariamro@usc.edu);
- or -713-563-2363 (Dr. Woodward will coordinate labels & contact with USC).
-or - the Kuhn Lab (kuhnlab@usc.edu) or Dr Peter Kuhn pkuhn@usc.edu

FEDEx SHIPPING INSTRUCTIONS

Packages must be shipped the same day of sample collection to ensure processing within 24 hours of collection time. Please select:

1. **Priority Overnight**
 2. **No signature required**
 3. **Email Notifications going to kuhnlab@usc.edu** as recipient. Select all notifications types: ship, tendered, exception, and delivery.
- Samples collected on Monday to Thursday must be shipped to:
USC, Kuhn Laboratory
3430 S. Vermont Ave., TRF 125
Los Angeles, CA 90089
Phone: 213-821-3051
 - Samples collected on Friday must be shipped for Saturday delivery and held for pick up at a local FedEx location. Please request the name of the Saturday on-call person to kuhnlab@usc.edu in order to address the package appropriately.
USC, Kuhn Laboratory
Attn: (On-call person)
840 Childs Way
Los Angeles, CA 90089
Phone: 213-746-4234

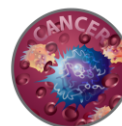
Email Notification (VERY IMPORTANT)

E-mail the Kuhn Lab (kuhnlab@usc.edu) the same day samples are collected and shipped. The e-mail must contain the following information:

1. Sample ID and type of sample (blood, bone marrow aspirate, tissue touch preparation)
2. # of tubes
3. # of tissue touch prep slides if applicable
4. Date and time of sample collection
5. FedEx tracking number

QUESTIONS?

Please contact Mariam Rodriguez Lee (mariamro@usc.edu; 858-405-9347).



NRG-BR002 Special Standard71 Kit Shipping Instructions Insert 3.31.16

- 1) Follow instructions in Section 10 of the protocol. Draw the blood into the special “Streck tube” and gently mix. Keep at room temperature.
- 2) Activate the PakSense as per the instructions in Steps 1-4 on the Orange Temperature Monitoring card. The PakSense monitors the temperature of the samples during transit. The instructions are located on the bright orange card inside the shipment and copied here.
 - Step 1: Fill out the origination detail
 - Step 2: Unpack the Paksense, Press and Hold the start button on label for two seconds until green light flashes.
 - Step 3: Peel cover off glue dot on back of label and stick the back label on the card
 - Step 4: Place orange card in protective sleeve, stick this to the outside of shipping box.
- 3) Place the PakSense in the slot inside the shipping cassette with the glue dot side down.
- 4) Place Streck tube with room temperature Blood for Plasma inside secondary containers and place tape over the lid
- 5) Place the two plastic containers next to each other (one empty, one with streck tube) on top of the Pak sense in one side of the cassette



- 6) Place into the insulated pouch, close pouch and place in outer shipping box.
- 7) Please Shipping label addressed to Dr Kuhn’s lab and the completed Orange Temp Monitoring Card on outside of box
- 8) Notify Dr Kuhn’s lab (kuhnlab@usc.edu) that the package is on the way and provide tracking information.

INSTRUCTIONS FOR USE

INTENDED USE

Cell-Free DNA BCT® is a direct draw whole blood collection tube intended for collection, stabilization and transportation of cell-free plasma DNA. This device also stabilizes and preserves cellular genomic DNA present in nucleated blood cells and circulating epithelial cells (tumor cells) found in whole blood. **This product has not been cleared by the U.S. Food and Drug Administration for In Vitro Diagnostic use. The product is For Research Use Only. Not for use in diagnostic procedures.**

SUMMARY AND PRINCIPLES

Accurate analysis of cf-DNA can be compromised by sample handling, shipping and processing, causing lysis of nucleated blood cells and subsequent release of cellular genomic DNA. Additionally, degradation of cf-DNA due to nuclease activity can be problematic.

The formaldehyde-free preservative reagent contained in Cell-Free DNA BCT^{1,2} stabilizes nucleated blood cells, preventing the release of cellular genomic DNA, and inhibits nuclease mediated degradation of cf-DNA, contributing to the overall stabilization of cf-DNA³. Samples collected in Cell-Free DNA BCT are stable for up to 14 days at temperatures between 6 °C-37 °C, allowing convenient sample collection, transport and storage⁴.

The formaldehyde-free preservative reagent contained in Cell-Free DNA BCT stabilizes circulating epithelial cells (tumor cells) in whole blood for up to 4 days at temperatures between 15 °C-30 °C⁵.

REAGENTS

Cell-Free DNA BCT contains the anticoagulant K₂EDTA and a cell preservative in a liquid medium.

PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Do not freeze specimens collected in Cell-Free DNA BCT as breakage could result.
3. Do not use tubes after expiration date.
4. Do not use tubes for collection of materials to be injected into patients.
5. Product is intended for use as supplied. Do not dilute or add other components to Cell-Free DNA BCT.
6. Overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytic results or poor product performance.

CAUTION

- a. Glass has the potential for breakage; precautionary measures should be taken during handling.
 - b. All biological specimens and materials coming in contact with them are considered biohazards and should be treated as if capable of transmitting infection. Dispose of in accordance with federal, state and local regulations. Avoid contact with skin and mucous membranes.
 - c. Product should be disposed with infectious medical waste.
 - d. Remove and reinsert stopper by either gently rocking the stopper from side to side or by grasping with a simultaneous twisting and pulling action. A "thumb roll" procedure for stopper removal is NOT recommended as tube breakage and injury may result.
7. SDS can be obtained at www.streck.com or by calling 800-843-0912.

STORAGE AND STABILITY

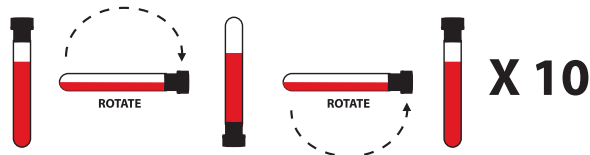
1. When stored at 18 °C-30 °C, unused Cell-Free DNA BCT is stable through expiration date.
2. Do not freeze unfilled Cell-Free DNA BCT. Proper insulation may be required for shipment during extreme temperature conditions.
3. Blood samples collected in Cell-Free DNA BCT for cf-DNA analysis are stable for 14 days when stored between 6 °C-37 °C.
4. Blood samples collected in Cell-Free DNA BCT for genomic DNA analysis are stable for 14 days when stored between 6 °C-37 °C.
5. Blood samples collected in Cell-Free DNA BCT for circulating epithelial cells (tumor cells) are stable for 4 days when stored between 15 °C-30 °C.

INDICATIONS OF PRODUCT DETERIORATION

1. Cloudiness or precipitate visible in reagent of unused tube.
2. If indications of product deterioration occur, contact Streck Technical Services at 800-843-0912 or technicalservices@streck.com.

INSTRUCTIONS FOR USE

1. Collect specimen by venipuncture according to CLSI H3-A6⁶.
Prevention of Backflow - Since Cell-Free DNA BCT contains chemical additives, it is important to avoid possible backflow from the tube.
 To guard against backflow, observe the following precautions:
 - a. Keep patient's arm in the downward position during the collection procedure.
 - b. Hold the tube with the stopper in the uppermost position so that the tube contents do not touch the stopper or the end of the needle during sample collection.
 - c. Release tourniquet once blood starts to flow in the tube, or within 2 minutes of application.
2. Follow recommendations for order of draw outlined in CLSI H3-A6⁶. Cell-Free DNA BCT can be drawn after the EDTA tube and before the Glycolytic inhibitor tube.
3. Fill tube completely.
4. Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate test results. One inversion is a complete turn of the wrist, 180 degrees, and back per the figure below:



5. After collection, transport and store tubes within the recommended temperature range.

DNA EXTRACTION

Extraction of cell-free plasma DNA and cellular genomic DNA can be accomplished using most commercially available kits that include a Proteinase K treatment step.

Cell-Free Plasma DNA

- Step 1. To separate plasma, centrifuge whole blood at 300 x g for 20 minutes at room temperature.
- Step 2. Remove the upper plasma layer and transfer to a fresh tube.
- Step 3. Centrifuge the plasma at 5000 x g for 10 minutes.
- Step 4. Isolate cell-free DNA per kit manufacturer instructions.

For optimal results, include a Proteinase K treatment step (≥30 mAU/mL digest) at 60 °C in the presence of chaotropic salts for 1 hour when extracting cell-free DNA.

Cellular Genomic DNA

- Step 1. To separate the white blood cells, either lyse the red blood cells and wash, or centrifuge whole blood and collect the buffy coat layer.
- Step 2. Isolate genomic DNA per kit manufacturer instructions.

For optimal results, include a Proteinase K treatment step (≥ 30 mAU/ml digest) at 60 °C in the presence of chaotropic salts for 2 hours when extracting cellular genomic DNA.

Note:

1. Cell-Free DNA BCT does not dilute blood samples; therefore, no dilution factor correction is necessary.
2. As in the case with most clinical laboratory specimens, hemolysis, icterus and lipemia may affect the results obtained on blood samples preserved with Cell-Free DNA BCT.

LIMITATIONS

1. For single use only.
2. Samples drawn in other anticoagulants or preservatives may cause coagulation in Cell-Free DNA BCT.
3. The use of butterfly collection needles for venipuncture is not recommended with the 10ml Cell-Free DNA BCT.

REFERENCES

1. Das K., Dumais J., Basiaga S., Krzyzanowski G. Carbon-13 nuclear magnetic resonance analysis of formaldehyde free preservatives. *Acta Histochemica* 2013; 115: 481-486.
2. Das K., Fernando M.R., Basiaga S., Wigginton S., Williams T. Effects of a novel cell stabilizing reagent on DNA amplification by PCR as compared to traditional stabilizing reagents. *Acta Histochemica* 2014; 116: 55-60.
3. Norton S.E., Lechner J.M., Williams T., Fernando, M.R. A stabilizing reagent prevents cell-free DNA contamination by cellular DNA in plasma during blood sample storage and shipping as determined by digital PCR. *Clinical Biochemistry* 2013; 46: 1561-1565.
4. Norton S.E., Luna K.K., Lechner J.M., Qin J., Fernando M.R. A new blood collection device minimizes cellular DNA release during sample storage & shipping when compared to a standard device. *Journal of Clinical Laboratory Analysis* 2013; 27: 305-311.
5. Qin J., Alt J.R., Hunsley B., Williams T., Fernando M.R. Stabilization of circulating tumor cells in blood using a collection device with a preservative reagent. *Cancer Cell International* 2014, 14:23.
6. Clinical and Laboratory Standards Institute. H3-A6, Procedures for the collection of diagnostic blood specimens by venipuncture. Approved Standard - Sixth Edition.

ORDERING INFORMATION

Please call our Customer Service Department toll free 800-228-6090 for assistance. Additional information can be found online at www.streck.com.

GLOSSARY OF HARMONIZED SYMBOLS

Batch Code	Biological Risk	Catalog Number	Use By	Manufacturer	Consult Instructions For Use	Temperature Limitation
Glossary of symbols may contain symbols not used in the labeling of this product.						

See www.streck.com/patents for patents that may be applicable to this product.



Streck
7002 S. 109 Street, La Vista, NE 68128 USA

350547-8
2016-02

Activating & Applying PakSense™ Labels

Step 1:

Fill out the origination detail.

Step 2:

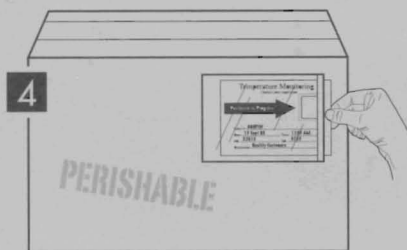
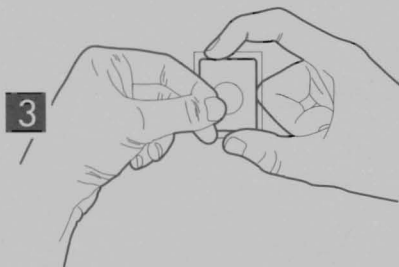
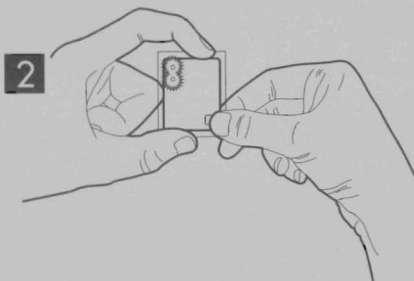
Press and Hold the Start button on label for two seconds until green lights flash.

Step 3

Peel cover off glue dot on back of label, stick to front of paper.

Step 4

Place paper in protective sleeve; stick pouch to packaging



Activación y colocación de las etiquetas PakSense™

1. Complete los detalles de origen en el frente
2. Presione por dos segundos el botón rojo de inicio en la etiqueta hasta que las luces destellen
3. Retire la cubierta del círculo adhesivo detrás de la etiqueta, péguela en el frente del papel
4. Coloque el papel dentro de la cubierta protectora, pegue la bolsa al paquete

PAKSENSE®

www.paksense.com
support@paksense.com
+1 208-489-9010

P/N: LITUNFIN
Rev Date: 08/2014



NRG Oncology

Specimen Transmittal Form
Special Streck Blood Tubes

Study# NRG-BR002

Case #

PLACE LABEL HERE

Institution

Institution No.

Participant's Initials

Participant's I.D. No.

INSTRUCTIONS: This form must be completed and mailed with the specimens upon submission. Please see protocol for list of required materials. Use one separate form for each timepoint.

STATUS	SPECIMEN OBTAINED DATE	TIME POINTS (per section 10 of the protocol)	*SPECIMEN TYPE (see table below)	NUMBER OF SPECIMENS	STORAGE METHOD	PATHOLOGY ACCESSION# / PT ID#
1	____ - ____ - ____ MM DD YYYY	T-0	99 - Streck Plasma			
4	____ - ____ - ____ MM DD YYYY	Arm - 1 3 months	99 - Streck Plasma			
4	____ - ____ - ____ MM DD YYYY	Arm - 2 4 weeks	99 - Streck Plasma			
	____ - ____ - ____ MM DD YYYY					
	____ - ____ - ____ MM DD YYYY					
	____ - ____ - ____ MM DD YYYY					

----- STATUS -----			
1	Pre-treatment	6	Recurrence
2	Surgical treatment	7	Autopsy
3	During treatment	99	Unknown
4	Post-treatment		

ENCLOSURES:

_____ Specimens - Streck Plasma

_____ This Submission Form

SEND TO:

FedEx/Courier address

Peter Kuhn
c/o Kuhn Lab at USC
3430 S. Vermont
Suite #106
Los Angeles, CA 90089

pkuhn@usc.edu

***SPECIMEN TYPE**

- 10 H & E Stained Slides (P2)
- 11 Paraffin Blocks (JB)
- 12 Punch Bx (JH)
- 13 Unstained Slides (JN)
- 14 Fresh Tissue (JF)
- 30 Whole Blood (JW)
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- 50 Urine (JU)
- 51 Saliva (JL)
- 52 Buccal Scrapings (JR)
- 60 CSF (JC)
- 99 Other, specify (JT)

Check all that apply.

Patient consents to:

- 1 Current research as specified in the protocol
- 2 Cancer research
- 3 Medical research
- 4 Being contacted about future research

SUBMITTED BY: _____

TELEPHONE NO: (_____) _____ EMAIL: _____