

BioSpecimen Exchange for Neurological Disorders (BioSEND)

The Late Effects of TBI (LETBI)


Training Webinar- LETBI

BioSEND Training Webinar Overview

1. Study Reminders
2. Site Equipment
3. Biospecimen Collection Protocol
4. Study Visit Protocol
5. Kits & Samples
 - Requesting Kits
 - Labels
 - Sample Collection & Processing
 - Sample Shipment
5. BioSEND Website
6. Contact Information

Study Reminders

Please remember...

- Biospecimens are limited, valuable resources.
- Standardization and quality are key! 
- Reference the BioSEND Manual of Procedures as needed.
- Do not replace or supplement any kit components without first receiving approval from BioSEND.

Site Equipment

The following items are to be supplied by the site:

- Personal protective equipment
- Alcohol prep pads
- Butterfly needles and hubs
- Tourniquet
- Gauze pads
- Bandages
- Sharps bin and lid
- Microcentrifuge tube rack
- Test tube rack
- Crushed ice
- -80°C Freezer
- Dry ice

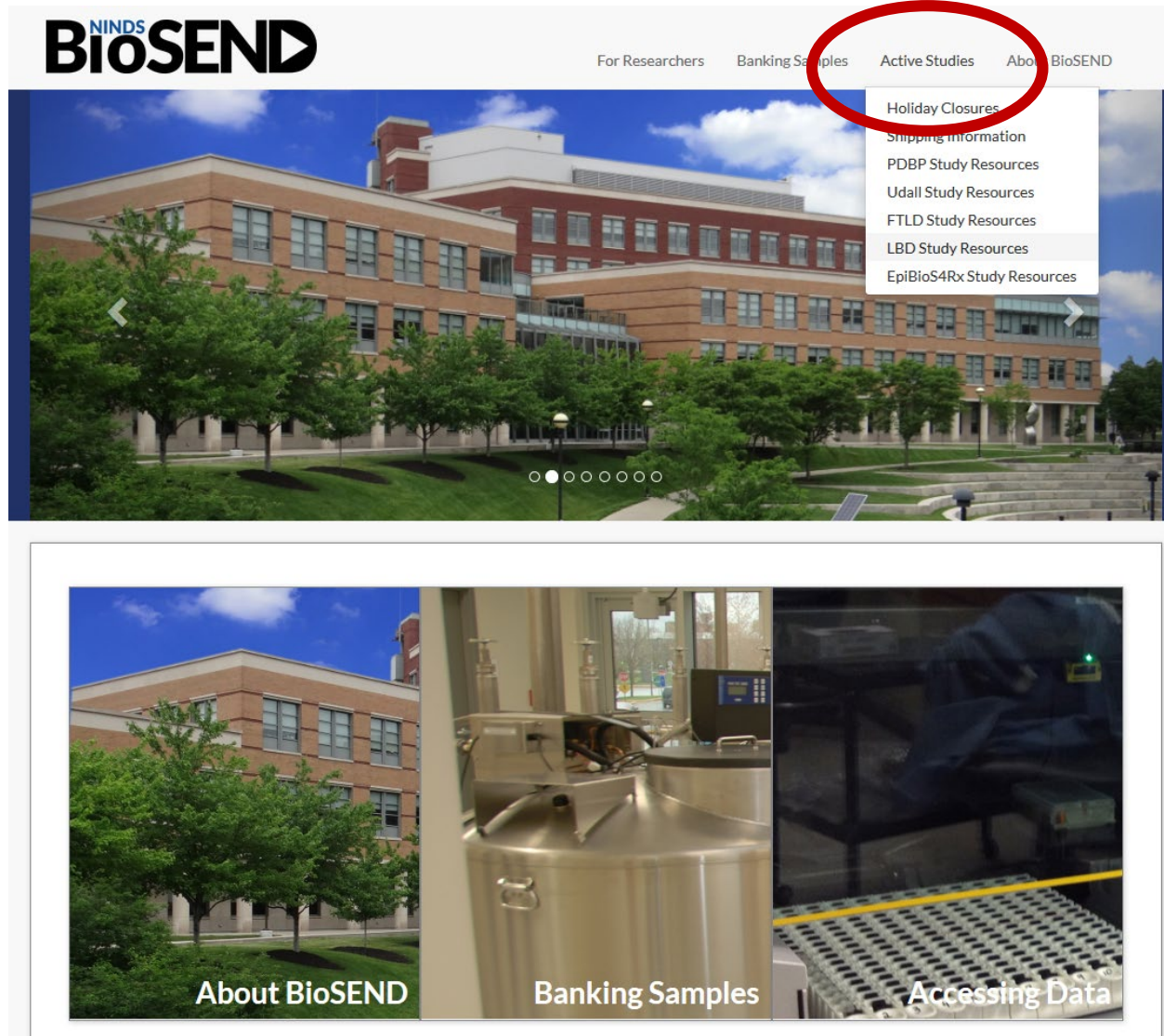
Biospecimen Collection Protocol

All specimen types FROZEN

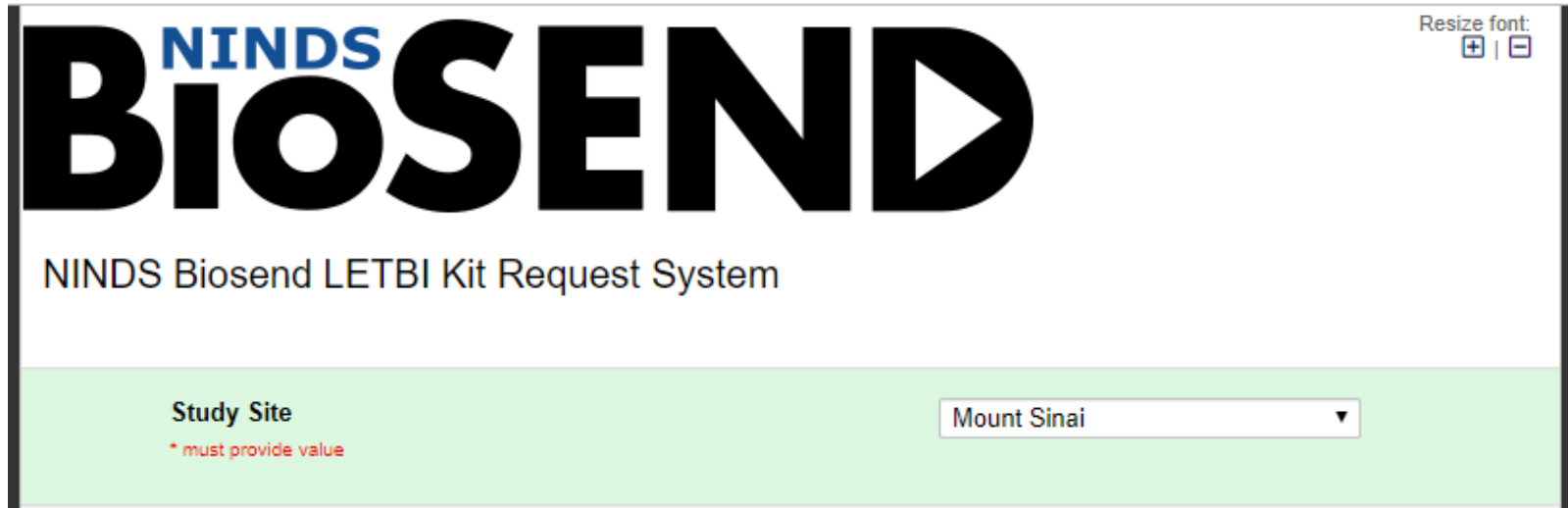
	V0	V1	V2
Plasma (6 x 1.5ml)	X	X	X
Buffy Coat (2 aliquots)	X	X	X

Requesting Kits

NINDS BioSEND Website



BioSEND Kit Request Module



The screenshot shows the NINDS BioSEND LETBI Kit Request System interface. At the top, the logo "NINDS BioSEND" is displayed in large, bold letters, with "NINDS" in blue and "BioSEND" in black. Below the logo, the text "NINDS Biosend LETBI Kit Request System" is visible. In the top right corner, there is a "Resize font:" option with plus and minus icons. The main form area has a light green background. It contains a label "Study Site" with a red asterisk and the text "* must provide value" below it. To the right of the label is a drop-down menu currently showing "Mount Sinai".

- <http://kits.iu.edu/biosend/letbi>
- **Choose your site from the drop-down list.**

BioSEND Kit Request Module

NINDS Biosend LETBI Kit Request System

Study Site

* must provide value

Mount Sinai ▼

Mount Sinai

Annell Ovalles
Brain Injury Research Center
Department of Rehabilitation and Human Performance
One Gustave L. Levy Place
Box 1163
New York, NY 10029-6574
212-241-5152
annell.ovalles@mountsinai.org
Enna.Selmanovic@mountsinai.org
Alara.Akyatan@mountsinai.org

Confirm site information:

- Study site
- Shipping address
- Contact name
- Email

Is the contact name above correct?

* must provide value

☐ Yes
☐ No

reset

Is the shipping address above correct?

* must provide value

☐ Yes
☐ No

reset

Is the e-mail address above correct?

* must provide value

☐ Yes
☐ No

reset

BioSEND Kit Request Module

Is the contact name above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
New Contact Name <small>* must provide value</small>	<input type="text"/>	
Is the shipping address above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
New Shipping Address <small>* must provide value</small>	<div><input type="text"/></div> <div>Expand</div>	
Is the e-mail address above correct? <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	reset
New e-mail Address <small>* must provide value</small>	<input type="text"/>	

- Is the information correct?
- Provide the correct information if needed

BioSEND Kit Request Module: Kit Type

Kit Type
****Please allow two weeks for shipment****
* must provide value

☐ Baseline Visit Kit
☐ Annual Visit Kit
☐ Supplemental Kit
☐ Extra Supplies

Please specify in comments if you need kits before the standard two week shipment time.


- Multiple kit types

BioSEND Kit Request Module: Baseline Kit

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input checked="" type="checkbox"/> Baseline Visit Kit <input type="checkbox"/> Annual Visit Kit <input type="checkbox"/> Supplemental Kit <input type="checkbox"/> Extra Supplies <small>Please specify in comments if you need kits before the standard two week shipment time.</small>
Baseline Visit Kit Quantity <small>* must provide value</small>	<input type="text"/>

- BioSEND creates ST numbers for baseline kits
- ST#s serve as the biorepository subject identifier
- Enter kit quantity

BioSEND Kit Request Module: Annual Visits

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input type="checkbox"/> Baseline Visit Kit <input checked="" type="checkbox"/> Annual Visit Kit <input type="checkbox"/> Supplemental Kit <input type="checkbox"/> Extra Supplies <small>Please specify in comments if you need kits before the standard two week shipment time.</small>
Annual Visit Kit Quantity <small>* must provide value</small>	<input type="text" value="1"/> <small>If requesting annual kits, you must provide the ST-numbers of all kits needed. Not doing so will cause delay in shipment, as we are unable to complete your request without this information.</small>
<p>If you going to request more than 10 kits/labels or prefer to upload a file with the Biorepository ID and Visit, you may download this template file and fill in the relevant information.</p> <p>Attachment:  BioSEND_Kit_IDs_Template.xlsx (0.01 MB)</p>	
<p>If you are using the provided template to upload Biorepository IDs and Visits, please upload your template file here.</p> <p>Upload document</p>	
1st Kit Visit ID (only if not using file upload option)	<input type="text"/> <small>e.g. ST-00012345</small>
1st Kit Visit Month	<input type="text" value="▼"/>
Comments	<input type="text"/>

- Please provide ST# and visit type for follow-up kits. We cannot complete your request without this information.

BioSEND Kit Request Module: Supplemental Kit

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input type="checkbox"/> Baseline Visit Kit <input type="checkbox"/> Annual Visit Kit <input checked="" type="checkbox"/> Supplemental Kit <input type="checkbox"/> Extra Supplies <small>Please specify in comments if you need kits before the standard two week shipment time.</small>
Supplemental Kit Quantity <small>* must provide value</small>	<input type="text" value="1"/>
Comments	<div></div> <div>Expand</div>
Each Supplemental Kit Contains: 10 - Purple-top EDTA tubes (Plastic, 10 ml) 10 - Purple cryogenic vials (2 ml) 10 - Grey cryogenic vials (2 ml) 10 - Disposable transfer pipettes (3ml) 2 - Cryobox, 25-slot 5 - Biohazard bag with absorbent sheet 5 - Shipping label packet (incl. waybill)	

- Contains a variety of extra kit components

BioSEND Kit Request Module: Extra Supplies

Kit Type **Please allow two weeks for shipment** <small>* must provide value</small>	<input type="checkbox"/> Baseline Visit Kit <input type="checkbox"/> Annual Visit Kit <input type="checkbox"/> Supplemental Kit <input checked="" type="checkbox"/> Extra Supplies
<small>Please specify in comments if you need kits before the standard two week shipment time.</small>	
Resealable Tube Pouches	<input type="radio"/> 2 <input type="radio"/> 4 reset
Cryobox	<input type="radio"/> 2 <input type="radio"/> 4 reset
Cryogenic Vial (2 ml) - Grey	<input type="radio"/> 10 <input type="radio"/> 20 reset
Cryogenic Vial (2 ml) - Purple	<input type="radio"/> 10 <input type="radio"/> 20 reset
FedEx® return Airbill	<input type="radio"/> 2 <input type="radio"/> 4 reset
Shipping Container for Dry Ice Shipments (includes shipping labels & airbill)	<input type="radio"/> <input type="radio"/>

- Allows you to choose specific supplies and particular quantities

BioSEND Kit Request Module: Multiple Orders

Kit Type

****Please allow two weeks for shipment****

* must provide value

☒ Baseline Visit Kit

☒ Annual Visit Kit

☒ Supplemental Kit

☒ Extra Supplies

Please specify in comments if you need kits before the standard two week shipment time.

- You can order more than one type of kit in a single kit request

BioSEND Kit Request Module: Submit

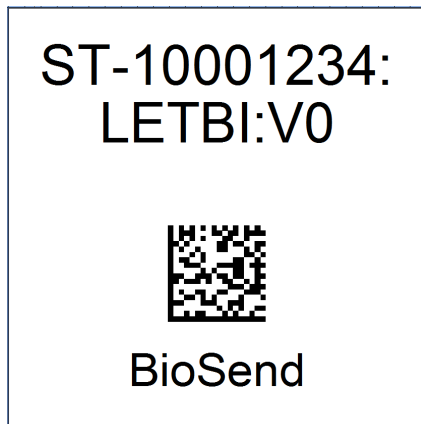
- Click “Submit” to turn in your request.
- The BioSEND staff will notify you that your request has been received and address any issues.

A screenshot of the NINDS Biosend PHD3 Kit Request System web form. The form is titled "NINDS Biosend PHD3 Kit Request System" and includes a "Resize font" option in the top right. The form fields are as follows: "Study Site" (a dropdown menu showing "Paulsen, Jane" with a red asterisk and "must provide value" below it); "University of Iowa" and "Jane Paulsen" (text fields); "Is the contact name above correct?" (radio buttons for "Yes" and "No" with a red asterisk and "must provide value" below it, and a "reset" link); "Is the shipping address above correct?" (radio buttons for "Yes" and "No" with a red asterisk and "must provide value" below it, and a "reset" link); "Is the e-mail address above correct?" (radio buttons for "Yes" and "No" with a red asterisk and "must provide value" below it, and a "reset" link); "Kit Type" (checkboxes for "Baseline Visit Kit", "Annual Visit Kit", "Supplemental Kit", and "Extra Supplies" with a red asterisk and "Please allow two weeks for shipment**" below it, and a note "Please specify in comments if you need kits before the standard two week shipment time."); and "Comments" (a large text area with an "Expand" link). The "Submit" button at the bottom is circled in red.

Labels

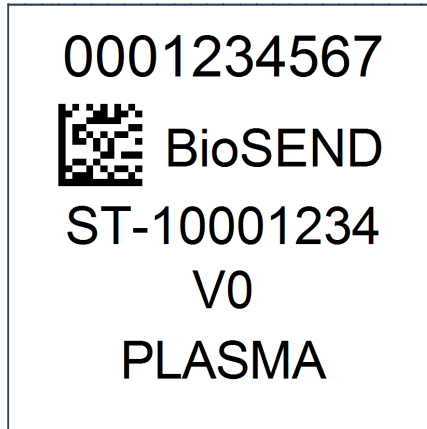
Types of Labels

Case Label



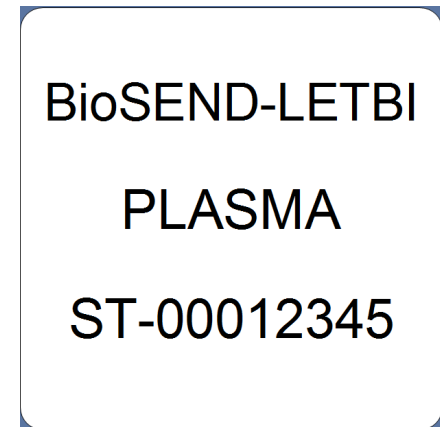
Identify study and Visit

Collection Tube Label



For collection tubes

Aliquot Label



For cryovials; will come
pre-labeled

Case Label

ST-10001234:
LETBI:V0



BioSend



Subject Number



Study and Visit



Biorepository Name

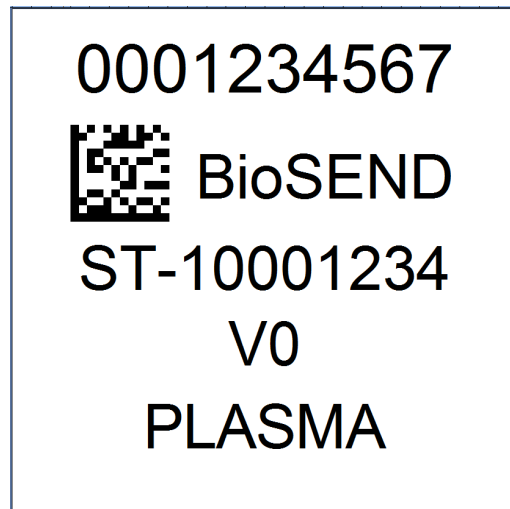
Case Labels

Case labels are placed:

- On the plastic biohazard bag of the cryovial transport box.
- *On the lid of frozen shippers*



Collection Tube Label



← **Biospecimen Number**

← **Subject Number**

← **Visit Type**

← **Specimen Type**

Aliquot Tube Label

BioSEND-LETBI

PLASMA

ST-00012345



Study Abbreviation



Specimen Type

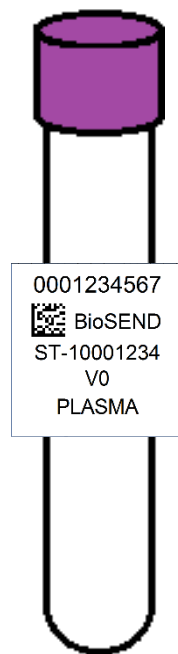


Subject Number

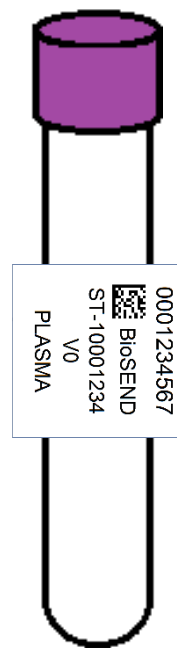
*Note: The tube itself will have a unique barcode printed in both 2D format (on bottom of tube) and human readable format (alongside of tube).

Collection Tube Labels

Collection Tube Labels are placed on all collection tubes



Incorrect



Correct

Aliquot Labels

Cryovials will be pre-labeled, but please verify that you are using the correct cryovial for the correct specimen type

Grey cryovials are used for
Buffy Coat aliquots



BioSEND-LETBI
BUFFY COAT
ST-10001234

Purple cryovials are used for
plasma aliquots



BioSEND-LETBI
PLASMA
ST-00012345

Sample Collection & Processing

Reminders:

- Collection of biospecimen blood samples should be from subjects who have been fasting for 8 hours or more. If fasting is not feasible, follow suggested low-fat diet.
- G force \neq RPM
- All specimens should be frozen and stored UPRIGHT
 - For plasma and buffy coat aliquots, please freeze samples upright in the cryobox provided

Calculating Centrifugation Speed

<https://www.eppendorf.com/CA-en/centrifuge-speed-calculator/>

Centrifuge Calculator

Rotational Speed and centrifugal force

To calculate the relative centrifugal force at the given rotor speed and given rotor radius, please enter the values in the appropriate fields and press the Calculate RCF key.

The Calculate Speed key provides information on the required rotational speed at the given relative centrifugal force and the given rotor radius.

Please specify centrifuge and rotor or enter rotor's radius directly

5810/5810 R

Rotors for 5810/5810 R

- 30-place fixed-angle rotor for 1.5-2.0 ml tubes (F-45-30-11)
- 30-place Aerosol-tight fixed-angle rotor for 1.5-2.0 ml tubes (FA-45-30-11)
- 6 x 85 ml High-speed fixed-angle rotor (F-34-6-38)

= Radius 10 cm

Relative centrifugal force (RCF)


RCF 1500 x g

Calculate Speed >>

<< Calculate RCF

Speed

Speed 3663 rpm



****The 3663 rpm speed was calculated using a hypothetical radius of 10 cm and a RCF of 1500 x g.***

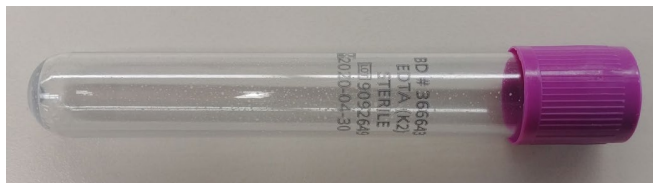
Specimen Collection

Tubes: 2 x 10 ml (lavender top) blood collection for plasma and buffy coat

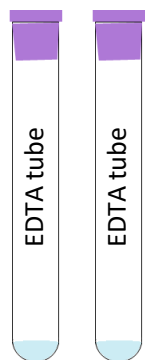


**EDTA
(Plasma)**

Plasma Preparation –10 ml EDTA (Purple Top) Tube



Step One



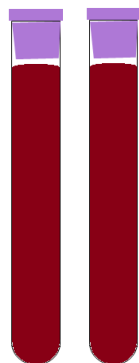
- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two



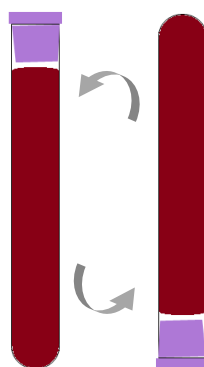
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three



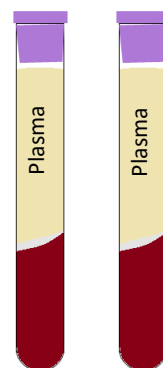
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



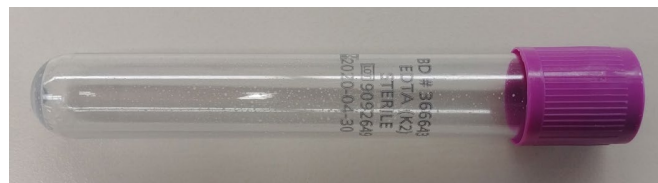
- Within 30 minutes of blood draw, centrifuge sample at 1500 x g at room temperature for 15 minutes.

Step Six

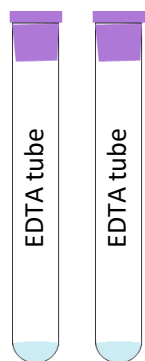


- Using a clean transfer pipet, aliquot 1.5 ml plasma into each purple cryotube.
- Store plasma aliquots upright at -80°C until shipment.
- See Appendix C for instructions on buffy coat collection.

Buffy Coat Preparation –10 ml EDTA (Purple Top) Tube



Step One



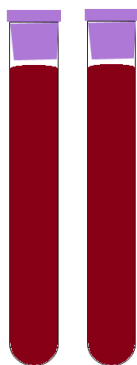
- Store tubes at room temperature.
- Label tubes with preprinted plasma labels prior to blood draw.

Step Two



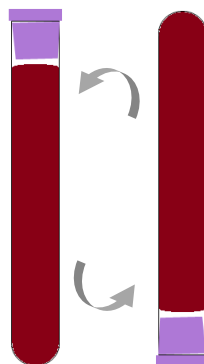
- Pre-chill cryotubes on wet ice for at least 5 minutes.

Step Three



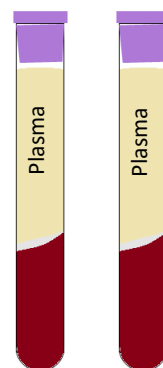
- Collect blood in EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Four



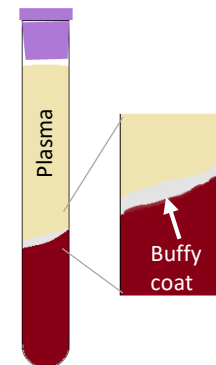
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Five



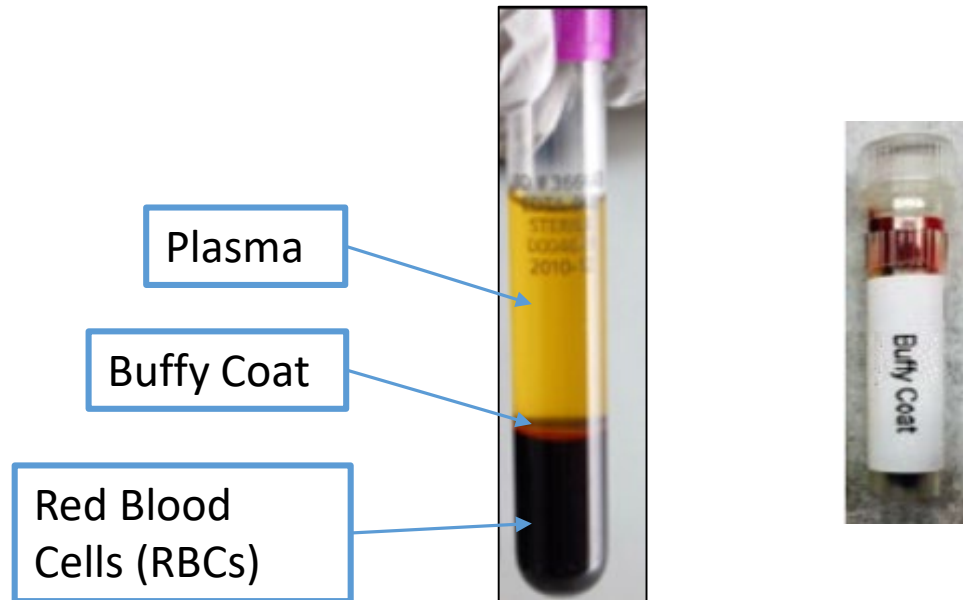
- Within 30 minutes of blood draw, centrifuge sample at 1500 x g at room temperature for 15 minutes.

Step Six



- Using a clean transfer pipet, collect the buffy coat (will have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into a grey cryotube.
- Store plasma aliquots upright at -80°C until shipment.

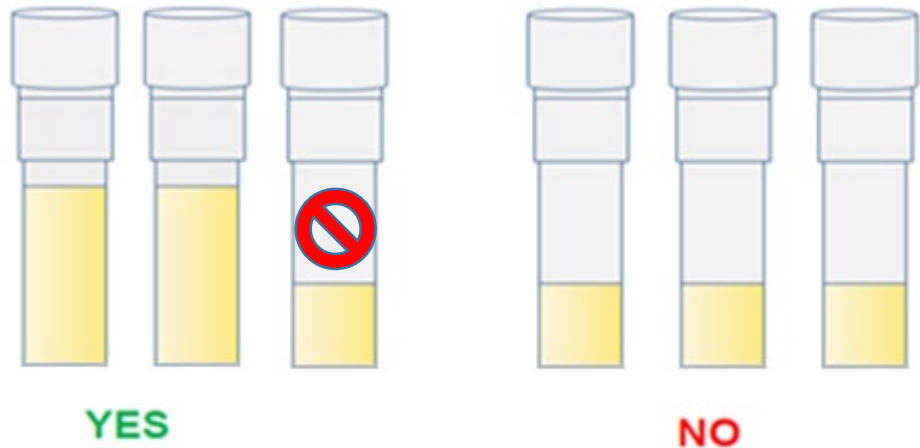
Buffy Coat Collection



Collect the buffy coat layer using the transfer pipet provided. Residual plasma as well as some RBCs will be included in this collection. A buffy coat will be reddish in color due to RBCs. Freeze buffy coats upright on dry ice or -80 freezer. Store in -80 freezer until shipment to BioSEND.

Plasma and Buffy Coat Aliquots

- Fill cryovials to 1.5ml
- Over-filled vials may burst in freezer
- Ship material to BioSEND
 - 6 Plasma aliquots
 - 2 Buffy Coat
- Do NOT send residual volumes to BioSEND



Blood Collection: Troubleshooting

Issue #1: Collection tube with little/no vacuum

- Always check expiration dates before beginning blood draw and discard expired tubes
 - *Tubes expire on last day of month printed on tube*
- Store tubes at ambient temperature
 - *Extreme temperatures can affect vacuum*
- Keep extra collection tubes from supplemental kit nearby during blood draw to replace “bad” tubes. These can also be requested through the Kit Request Module.
- If frequent occurrence, report tube type and lot numbers to Indiana University

Blood Collection: Troubleshooting

Issue #2: Hemolyzed (pink/red) plasma

Cause: Blood Collection Methods	Corrective Action
Improper venipuncture site	Draw from median cubital, basalic, and cephalic veins from antecubital region of arm
Prolonged tourniquet use	Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided
Not allowing alcohol to dry on skin before venipuncture	Without touching, allow the venipuncture site to air dry
Lumen of needle too close to inner wall of vein (indicated by slow blood flow)	
Use of too large/small bore needle resulting in excess force applied to blood	Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are 19 – 23.
Pulling/pushing plunger too fast while drawing/transferring blood	Avoid drawing the syringe plunger too forcefully when collecting blood
	Ensure all blood collection assemblies are fitted securely, to avoid frothing

Blood Collection: Troubleshooting

Issue #2: Hemolyzed (pink/red) plasma

Cause: Sample Processing Methods	Corrective Actions
Vigorous mixing/shaking	Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube
Not allowing serum to clot for recommended time	Serum tubes without clot activator should be allowed to clot for 60 min in a vertical position
Exposure to excessive heat or cold	Keep samples at ambient temp
Prolonged contact of serum/plasma with cells	Do not store uncentrifuged samples beyond recommended time

Reference: BD's "Tech Talk" newsletter, Vol. 2, No. 2, October 2003
(http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

Sample Shipment

Frozen Samples

- All samples are shipped frozen
 - Plasma and buffy coat
- Ship Monday-Wednesday Only via FedEx Priority Overnight
- Schedule FedEx® pickup
- Email Sample Record and Shipment Notification Form including FedEx® tracking number **AHEAD OF SHIPMENT**



Packaging and Shipping Frozen Samples



Place plasma and
buffy coats in cryobox



Place cryobox in
biohazard bag with
absorbent sheet,
seal, and label with
case label.

Packaging and Shipping Frozen Samples



Place approx. 2-3 inches
of dry in bottom of
shipper



Place biohazard bags upright in box



FILL dry ice to top
of box

Packaging & Shipping Troubleshooting

Issue: Broken/Damaged Tubes

Cause	Preventative Action
Over filling tubes	Fill tubes to suggested volume. If any sample still remains, place in an additional tube
Improper packaging	Ensure the tubes are securely placed into the bubble wrap pouch and are placed in a separate bag from the boxed plasma, serum, and CSF.
Rough shipping conditions	Extra bubble wrap may be needed to pad blood tubes
Extreme changes in temperature (ambient→freezer; freezer→dry ice)	Wrapping the tubes in bubble wrap before freezing may help slow the cooling process

Shipping Frozen Samples

- Hold packaged samples in a -80°C freezer until pickup.
- ***Samples should be received at BioSEND within 2 weeks of collection.***



Sample Shipment Notification Form

Sample Record and Shipment Notification

Study:

Site Name:

Principal Investigator:

Coordinator:

Telephone:

Email:

Please list only ONE subject per Sample Record Summary and Shipment Notification Form

GUID:

Subject ID (ST# from pre-printed labels):

Gender:

Visit Type:

Age in Years:

Plus Months:

Instructions: Ship Frozen Shipments Monday- Wednesday ONLY! Ambient Shipments (purple-top EDTA tube) may be shipped Monday- Thursday (preferably Monday- Wednesday) provided they are received at Indiana University within five days of collection. This form must be completed for shipment of all research samples. Notify Indiana University (email preferred) in advance of shipment using contact information below. Please also include a physical copy in the shipment box. **Ensure all frozen shipments are completely filled with dry ice.**

Date Sample(s) Shipped:

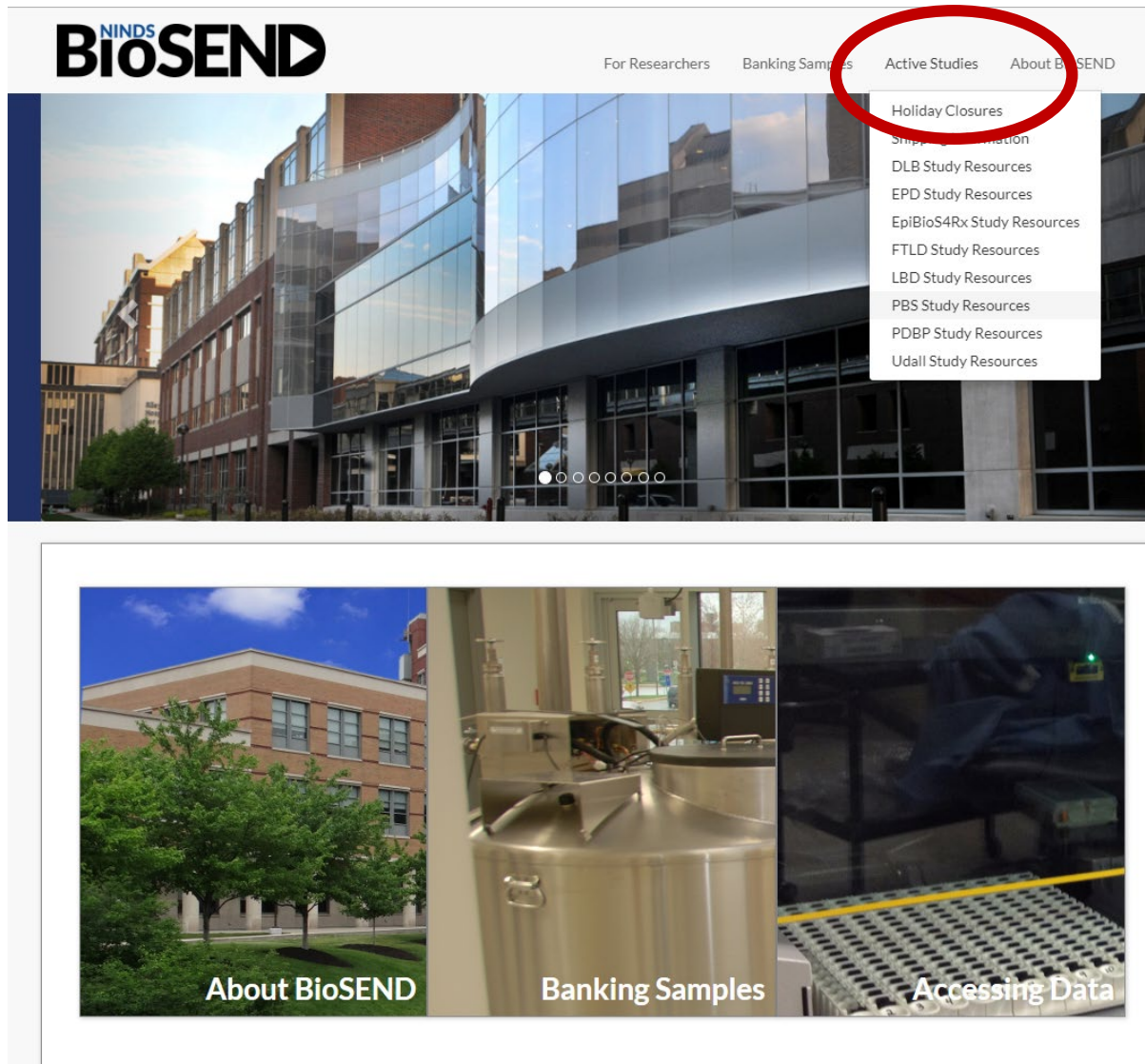
FedEx Tracking Number:

In the table below, please indicate the date of specimen collection and number of tubes/aliquots submitted.

Completed by Submitter/Site			
Dates of Draw	Specimen Type	Number of Tubes/ Aliquots sent to BioSEND	Notation of Problems
	DNA		
	RNA		
	Buffy Coat		
	Plasma		
	Serum		
	CSF		
	Whole Blood		

Contact Information: Indiana University; Email: biosend@iu.edu Ph: 317-278-0594

NINDS BioSEND Website



Holiday Closures

Date	Holiday
January 1	New Year's Day
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
July 4	Independence Day (observed)
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Note that BioSEND has extended closures surrounding the Thanksgiving, Christmas, and New Year's Holidays. BioSEND will send notification of these dates prior to the holiday season.

BioSEND Contact Information

- Questions?

Please contact: Claire Wegel (cwegel@iu.edu)

- Email: biosend@iu.edu

Questions?