

Guidance for Completing your NBB Tissue Request

Planning Your Tissue Request

- If you are not sure precisely what tissue, tissue regions, or quantities are best for your research project, please submit a request for help to the [NIH NeuroBioBank email address](#) before completing the on-line request.
- The Request Form requires you to provide a **detailed** rationale for 1) number of subjects requested, 2) amount of tissue requested per subject, and 3) number of brain regions requested. Please see the Acceptable Tissue Amount Guidelines (below) to plan your request.
- The more restrictive your subject characteristics, the less likely a brain bank will have tissue that meets your requirements. Ensure that any restrictions you place on subject characteristics (e.g., postmortem interval (PMI), RNA Integrity Number (RIN), age range) are necessary for your study to be valid.
- If you have not performed a pilot study in human tissue, your first request should be for tissue to perform a pilot study using your proposed methods.

Completing Your NBB Tissue Request Form

1. Complete all applicable fields. Required fields must be completed in order for the system to accept the request. Incomplete information will delay the review of your request, especially Specimen Shipping Information.
2. **Request Name** should be a short descriptor.
3. **Specimen Needs**
 - a. This component is divided into 2 sections: Fixed Tissue Request and Frozen Tissue Request. If you require both fixed and frozen tissues, please complete both sections, and note in the “Subject Specimen requirements” box if the fixed and frozen tissues need to come from the same subjects.
 - b. Each row should have one subject diagnostic category and one tissue type. For example, if you require Brodmann Area (BA) 9/46 and BA21 from Unaffected Control subjects and subjects with Major Depressive Disorder, the request would have 4 rows.
 - c. “Unaffected Control” are subjects with no known psychiatric or neurological diagnoses.
 - d. Please type out the name of the diagnostic category, and do not use abbreviations.
 - e. Minimum Tissue Size must follow the Acceptable Tissue Amount Guidelines (below). A detailed rationale must be provided for any deviations from these guidelines.
4. **Subject/Specimen requirements:** this section should include any additional requirements pertaining to the subjects/specimens themselves (e.g., PMI, RIN, fixation type, sex, etc). As noted above, the more restrictive your subject characteristics, the less

likely a brain bank will have tissue that meets your requirements. Ensure that any restrictions you place on subject characteristics are necessary for your study to be valid.

5. Request Details

- a. Title of Research Plan that will appear at the top of your MTA.
- b. Concisely describe the request according to the instructions. If you are requesting multiple anatomical regions per subject, the rationale must be clearly indicated here.
- c. List each type of assay or method to be used with the requested tissue. Include the amount of tissue needed for each assay/method per subject. For example, “qPCR, 2mg grey matter per subject.”
- d. Indicate whether you or your direct collaborators have used the method(s) proposed in this request with human postmortem tissue before.
 - i. If the answer is “No,” then this request should be for tissue for pilot studies.
- e. Provide a rationale for the number of subjects requested. Acceptable rationales include power analyses, or accepted standards in the field (with appropriate citations).

Determining Your Tissue Needs

The following table indicates acceptable amounts of tissue that can be requested for different types of studies. As different brain banks process and store their samples differently (see NBB Best Practices), you may be supplied with more tissue than you request. However, each of the tissue amounts below will provide enough material to perform each type of methodology listed. These values were derived in collaboration with researchers who have successfully performed each methodology using human brain tissue.

Method	Accepted Tissue Amount/Region/Subject
FACS sorting with NeuN	≤ 600mg of gray matter
HITS-CLIP	≤ 300mg
Immunoblot	≤ 10mg of tissue per protein examined
Immunohistochemistry	Sufficient tissue for ≤ 8 sections per condition <i>(May be provided as free floating sections, formalin-fixed paraffin-embedded sections, fixed tissue blocks, or paraffin-embedded tissue blocks dependent upon region requested and brain bank)</i>
Mass Spec:	
Simple Mass Spec with no enrichment or multidimensional fractionation	≤ 15mg
Mass Spec with sucrose density gradient based sub-cellular enrichment	≤ 350mg
Mass Spec with multidimensional separation with or without isobaric labeling tags (e.g., TMT or iTRAQ)	≤ 150mg
Co-IP with Mass Spec	50 – 500mg

Mass Spec for post-translational modifications	50 – 500mg
Microarray	≤ 5mg
qPCR	≤ 5mg
RNASeq	≤ 60mg

If your methodology is not listed, or you require more tissue than is indicated, please provide a detailed rationale supporting the amount of tissue you are requesting. Multiple brain regions such as hippocampal subfields, and thalamic and hypothalamic nuclei are small, and many investigators are interested in studying these regions. Ensure that your requests are anatomically possible before submitting. If you need guidance on this issue, please contact the NIH NeuroBioBank at neurobiobank@mail.nih.gov.