mammaprint®

targetprint®

blueprint™

theraprint™

agendia®

decoding cancer.

SYMPHONY™

Breast Specimen Sampling Instructions

1. Pre-surgery preparations

The fresh, unfixed tumor specimen must be delivered to pathology immediately following surgical removal.

A. Notify all parties involved in the surgical procedure, including pathology, that a tissue sample of the patient's fresh, unfixed tumor specimen will be required for Agendia's gene expression profiling tests (i.e. MammaPrint®, TargetPrint®, BluePrint™, TheraPrint®).

B. Confirm that a pathologist or other qualified person is available to obtain a fresh tissue sample from the tumor within 1 hour of surgical removal.

C. Confirm that the Agendia Specimen Collection and Transportation kit ("Specimen Kit") is readily available at the location where the specimen will be obtained.

Please do not place specimen in formalin until after the punch biopsy has been obtained for gene expression profile testing

2. Specimen collection and transportation kit

The sample must be obtained within 1 hour of surgical removal.

A. The specimen kit contains essential items for obtaining and preserving the tissue sample (for packaging and shipping instructions see Sections 7 and 8).

B. The specimen sample should be placed in the inner vial labeled RNARetain® (attached to the inside of the screw cap of the larger plastic tube).

C. The sampling tool may be used to obtain a punch biopsy sample from a surgical specimen. For instructions on how to use the punch biopsy tool see Section 4.

Note: Needle core biopsies, punch biopsies or excisional biopsies are acceptable.

3. Obtaining a sample from a surgical specimen

A. Preliminary gross examination is performed as needed and the surgical specimen is inked for assessment of margins.

B. Specimen is bisected or serially cross-sectioned to expose the tumor for Agendia tissue sampling. The sample may be excised with a scalpel or with the optional punch biopsy tool provided in the sampling kit.

   Note: Prevent extended exposure of the tumor surface to air to avoid air drying of tumor tissue. If necessary, keep wet with normal saline.

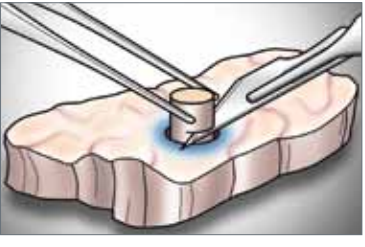
C. It may be valuable to place the surgical specimen in the freezer for 10-20 minutes to increase its firmness prior to obtaining the Agendia specimen.

D. Obtain the specimen:
   1. If using a scalpel, the tissue sample should be 3 to 4 mm in thickness (maximum of 4 mm) and between 5 and 10 mm in diameter. This size allows timely and thorough perfusion of the RNARetain® preservative.
   2. If using a punch biopsy tool, the tissue core will measure 3 mm in diameter and should be approximately 5 mm in length.
      a. For instructions on how to use the punch biopsy tool see Section 4.

   Note: There is a higher probability of the sample being analyzable if the punch tool is used.

E. Place the sample immediately into the RNARetain® specimen vial. The sample is stable in this preservative solution for 7 days at room temperature. Please ship the specimen to Agendia to arrive before the 7th day.

4. Instructions for using the punch biopsy tool



A. The punch tool is held between the thumb and index finger with its cutting end placed perpendicular to the tumor surface.

B. Under moderate pressure, roll the punch between the thumb and index finger in a screwing or rotary motion pressing gently into the tumor. Once a depth of approximately 5 mm has been reached, angle the punch tool at 45 degrees while still rotating in order to sever the tissue core at its base.

C. Withdraw the punch tool with the biopsy core contained within and extract the core with tweezers through the side window within the tip of the punch tool. Do not crush the tissue.

D. If the tissue core slips out of the punch tool because its base is still attached to the main specimen, the core should be carefully grasped and raised with tweezers and separated at its base with scissors or a scalpel. Do not crush the tissue.

E. If the tissue core is lodged within the punch tool chamber, eject the core by inserting a stylus type device down the chamber of the tool, gently pushing out the core.

F. Once the tissue sample has been obtained, place the sample immediately into the RNARetain® specimen vial.

5. Sampling tips and choosing the sampling site

Image 1

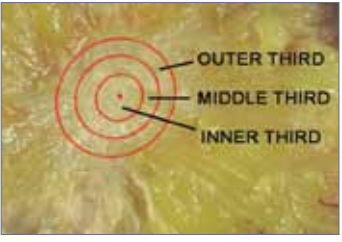


Image 2

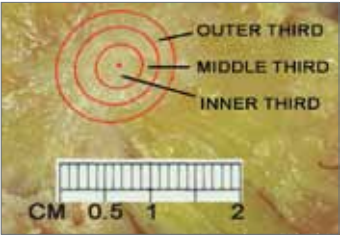


Image 3

A. The optimal biopsy site depends on the size of the tumor. As a general rule, do not take the sample from the periphery of the grossly identified tumor which often consists predominantly of peri-tumoral fibrous tissue with few cancerous components.
   1. For tumors larger than 1.5 cm in diameter, the sample should be taken from the middle third of the tumor. Avoid the inner third since the center of larger lesions may be necrotic.
   2. For tumors equal to or smaller than 1.5 cm in diameter, the sample should be taken from the inner two thirds of the lesion.

B. Avoid areas with obvious necrosis or hemorrhage.

C. If two distinctly separate lesions are identified, a biopsy of both lesions is recommended.

D. Very soft surgical specimens may be difficult to bisect without distorting the surface margins. These specimens may be "firmed up" by being chilled in the freezer for about 10 minutes after the outer margins have been inked. Be careful not to freeze the tissue.

E. Examples are shown in the three images to the left.

Image 1 *depicts a surgical specimen with a central poorly defined yellow-grey tumor.*

Image 2 *shows the tumor subdivided into three concentric zones, an inner third, a middle third and an outer third.*

Image 3 *shows an added ruler according to which the tumor measures approximately 1.3 - 1.5 cm in diameter. In this example, the biopsy should be taken from the inner two thirds of the surgical specimen.*

6. Recommendations for needle core biopsies

A. Needle core biopsies should be obtained with a 14 gauge or larger needle.

B. Please **provide three cores** to increase the probability of tumor-positive biopsies. In order to minimize sampling failures, one of the cores selected for the Agendia test should be the first or second core obtained. This is particularly important for small tumors of 1 cm or less in diameter.

   Note: The likelihood of finding cancer in needle core biopsies is directly proportional to the size of the tumor and the number of cores obtained. In a patient with invasive breast carcinoma (as proven by subsequent lumpectomy or mastectomy) the probability of finding cancer is about 95% if six cores are taken but decreases significantly with a lesser number of cores.

D. **Avoid tissue drying!** While successive needle biopsies are being obtained by the breast surgeon or radiologist, the biopsies already taken must not be left exposed to open air because drying artifacts and RNA degradation of these thin tissue strands may develop within minutes. The biopsy cores should be kept moist between two saline soaked Telfa pads until samples are selected for MammaPrint.

E. **Selection of cores for MammaPrint:** Avoid samples with obvious fatty tissue which has a very soft consistency and a translucent yellow tinge. Cancer tissue is typically firm and grey to grey-yellow in color, and often indistinguishable from areas with dense, firm but benign fibrous tissue.

7. Packaging the sample for shipment

Ship at room temperature; do not freeze the sample.

A. Place a barcode label from the completed requisition form onto the specimen vial.

B. Replace the small specimen vial into the larger specimen tube and place the tube into the specimen safety bag.

C. Place the sealed specimen bag into the shipping kit along with the completed test requisition form.

D. 1. Within the US, package the kit into the FedEx shipping pak and attach the pre-printed label for shipment to Agendia. Please call FedEx® at 800-goFedEx (800.463.3339) for specimen pickup.

2. For outside the US, put the specimen collection and transportation kit into the TNT pak. Please call your local TNT office to schedule a package pickup.

8. Completing the test requisition form

Please provide as much information as possible.

A. If you use our customer portal for ordering, please enter your test request at customers.agendia.com.

B. Record specimen collection date.

C. Complete the healthcare provider's information including the National Provider Identifier number (NPI) for US specimens. Include an email address and/or a fax number for where the Agendia test result(s) should be sent.

D. Complete patient information, including name and date of birth.

E. Ensure appropriate clinical information is provided, including the diagnosis code (ICD-9 code for US specimens).

F. Ensure payment option is selected in the method of payment section.

G. If the insurance billing option is being selected, ensure all patient contact information is complete, including telephone number and the following:
   1. Completely fill out insurance information section
   2. Legible photocopies of the front and back of insurance card(s)
   3. When applicable, patient's signed ABN or AOB
   4. If patient payment option (self pay) is selected, information must include either credit card information or check/money order

H. Ensure the ordering physician and/or submitting pathologist has signed and dated the form

I. Retain a copy of the test requisition form for your records

9. Results

You will receive the Agendia test result(s) within 10 working days by your specified means of reporting: email, fax, hard copy and/or secure intranet account once the patient's specimen is received at Agendia.

10. Questions

If you have any questions regarding the tumor sampling procedure, or if you require additional Agendia Specimen Kits, please contact Agendia or visit www.agendia.com.

US Specimens:

Email: customercare@agendia.com

Ph: 888.321.2732

Non-US Specimens:

Email: customerservice@agendia.com

Ph: +31 20 462 1510

11. Material Safety Data Sheet

Asuragen's RNARetain® MSDS: Per OSHA 29CFR1910.1200 and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC the following product does not require a Material Safety Data Sheet (MSDS). Therefore, Asuragen does not provide an MSDS for the following product.

Asuragen's RNARetain® does not contain more than 1% of a component classified as hazardous and does not contain more than 0.1% of a component classified as carcinogenic. However, when working with this or any chemical reagent, we recommend the use of gloves, laboratory coats, and eye protection. Asuragen assumes no liability for damage resulting from handling or contact with this product.

For in vitro Diagnostic Use.
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