Validation Of Antibodies For ChIP

I. Purpose

The purpose of this work instruction is to outline the protocols to validate an antibody, once it is received, for ChIP (Chromatin Immunoprecipitation).

II. References

Document Title	Document Number
N/A	N/A

III. Related Documents

Document Title	Document Number
Antibody Validation Western Blot	LIBPR.0076
Native ChIP Using 100,000 Cells	LIBPR.0138
MODified Histone Peptide Array	LIBPR.0071
Nimbus-assisted 96-well PCR –enriched Library Construction for Illumina Sequencing	LIBPR.0137

IV. Procedure

1. When to Order An Antibody

1.1. Follow the guidelines below to determine when to place an order for an antibody:

a. Less than 50 immunoprecipitations (IPs) worth of antibody remaining in production stock. This is referring to the diluted aliquots
b. Request of supervisor

1.2. Discuss with supervisor before placing an order. Request the current production lot of the antibody when entering the information on the ordering sheet. The ordering sheet can be found in geneexplab\Library Core\Lab Operations\Ordering. Purchasing Group will contact the vendor/manufacturer before placing the order if the current production lot is available. If it isn't, supervisor will contact the manufacturer and order 1 vial of the new lot for in-house validation.

2. Receiving/Storing and Barcoding the Antibody(ies)

2.1. Vial(s) of antibody are shipped on either dry ice, wet ice or at room temperature. The containers are placed in the 6th floor Library Core receiving area once received by the Purchasing team.

2.2. Remove the vial and the data sheet from the shipping container. Quickly check the data sheet for the lot# of the antibody. If the lot # is not listed, write the lot#, which is on the vial, on the data sheet. Also note the receiving date on the data sheet.

2.3. Transfer the vial to the -20°C freezer or 4°C, depending on what the manufacturer recommendation for optimal storage conditions and place the vial in the 'Antibodies to be Validated' box.

2.4. Your supervisor will generate a solution ID (sol#) in LIMS for the vial or pooled vials of antibody with the information from the data sheet. Antibody info such as concentration, clonality, special note, antibody volume etc. will also be entered by supervisor. This information will be tracked in 'Antibody Inventory Tracking' page in LIMS.

2.5. Place the sol# barcode on the antibody vial.

2.6. Scan the antibody into the 'Pending Validation' box (rac116433) located in the -20°C freezer or '4C Fridge' box (rac27643).

2.7. Note the solution ID on the data sheet and keep it in the Antibodies Binder as a reference.

3. Pooling Antibodies

3.1. If more than one vial of antibody type is received; the vials of antibodies are pooled by lot# if they are shipped within the same order. Physical pooling should be done when the antibody is to be validated by a process. This is to avoid repeat freeze thawing. Follow the criteria listed below to determine whether or not the vials should be pooled:

a. Same order, same shipment arrival, same Lot# - POOL
b. Same order, different shipment arrival, same Lot# - DO NOT POOL
c. Same order, same/different shipment arrival, different Lots# - DO NOT POOL

4. Processes to Complete to Validate the Antibody

4.1. The following three processes are to be completed to validate new antibody(s) received:

4.1.1. Histone Peptide Array (PA) using document LIBPR.0089 MODified Histone Peptide Array.

4.1.2. Western Blot Analysis (WB) using document LIBPR.0076 Antibody Validation Western Blot.

4.1.3. ChIP in HL60 cells using document LIBPR.0138 Native ChIP Using 100,000 Cells.

4.1.4. Library Construction using document LIBPR.0119 "96-Well PCR-enriched Library Construction for Illumina Sequencing" or LIBPR.0137 "Nimbus-assisted 96-well PCR –enriched Library Construction for Illumina Sequencing" and submission to sequencing.

4.2. The following criteria, shown in Figure 1, should be followed when a shipment of an antibody arrives when determining method of validation (Pooled Vials or Singlets).

Antibody	Western	Peptide Array	Native ChIP	LC including qPCR	Sequencing
Lot same as production	N	N	Y	Y	N
Lot different than production	Y	Y	Y	Y	Y
Monoclonal (same OR different lot as production)	N	N	Y	Y	N

Figure 1

5. Send out Results and Confirm Results With Supervisors

5.1. Summarize the results of the processes listed in Step 4 in a powerpoint presentation. Include all pertinent details such as type and amount of chromatin used to validate the antibody, gel images, Agilent profiles, and any technical issues encountered during the processes. If unsure, consult APC.

5.2. Send the powerpoint document via email to supervisors. Upon confirmation that the antibody has been passed and is suitable for production, proceed to the following step.

6. Dilute and Make Aliquots for Native ChIP

6.1. Dilutions are made based on the concentration of the antibody and the amount of the antibody that will be used in the IPs in the Native ChIP process.

6.2. Sample list of some production antibodies commonly used are in Figure 2 as well as the amount of antibody per IP.

Antibody	Supplier	Catalog #	Amt/IP (ug)
H3K4me1	Diagnode	pAb-037-050 (C15410037)*	0.5
H3K4me3	Cell Signaling	9751S	0.75
H3K9me3	Diagenode	pAb-056-050 (C15410056)*	0.5
H3K27me3	Diagenode	pAb-069-050 (C15410069)*	0.5
H3K36me3	Abcam Hiroshi Lab	ab9050 n/a	0.5
H3K27ac	Hiroshi Lab	n/a	0.5

*Alternative Catalogue Number

Figure 2

6.3. The concentration of the stock antibody(ies) is high; therefore it requires dilution for use in LIBPR.0138 Native ChIP Using 100,000 Cells as noted in Figure 2.

6.4. On ice, dilute the stock antibody to an appropriate working concentration. Dilute the antibody in the same buffer the stock is stored in. The storage buffer components are listed on the data sheet provided by the supplier.

6.4.1. Hiroshi H3K36me3 and H3K27ac are to be diluted fresh before use using the same storage buffer as used by antibodies from Diagenode.

6.5. Aliquot appropriate volume into 0.5mL non-stick tubes for 1x use. Ensure enough dead volume is added.

6.6. Create aliquots in LIMS. The volume of each aliquot is in ml not µl.

6.7. Put the barcodes on the tubes and store the aliquots in the -20ºC production boxes.