

Submission of external antibodies to be included in the Protein Atlas

Call for antibodies

Hereby, the Swedish Human Protein Atlas (HPA) program, funded by the (non-profit) Knut and Alice Wallenberg Foundation, invites submission of antibodies from both academic and commercial sources to be included in the human protein atlas (www.proteinatlas.org). All antibodies will be validated by the HPA-program by a standard procedure and antibodies that are accepted will be used in the tissue-profiling program to generate high-resolution immunohistochemistry images representing a wide spectrum of normal tissues and cancer types. During the pilot project phase, the validation will be done free of charge. For each antibody, 576 spots of human tissue from 360 different individuals are immunostained and manually annotated. Academic collaborators will have access to all biological data at least 6 months before the images will be published as part of the Human Protein Atlas. Commercial vendors will receive a direct web-link from the antibody information page on the Protein Atlas to a web-link decided by the antibody provider.

Submission of antibodies

The collaborator is asked to fill-in the excel-sheet form (HPA_submission_form-ab) with information, such as:

- Gene/protein Id of target (Ensembl Id)
- Description of target used for the antibody generation
- Description of type of antibody (pAb, mAb or recAb)
- Results from quality assurance (preferably a web-link but otherwise pdf-file)

The submissions are prioritized by the HPA Priority committee based on the extent of the validation and the presence or absence of the protein target in the HPA pipeline. For submissions that are approved, the collaborator will be asked to send in a vial (free of charge) of each antibody approved. A minimum amount of 50 µg or equivalent will be needed for each antibody.

Submissions could range from a single antibody to several hundreds of antibodies. Use one line in the excel sheet per antibody. Send the submission form (excel) to: kenneth.wester@genpat.uu.se

Use of the antibodies by the HPA program

Each antibody is tested according to HPA-standard quality assurance procedures, i.e., on Western blots and on a special designed tissue microarray (TMA) initially using two antibody-dilutions and heat epitope retrieval in citrate buffer. In selected cases, other pre-treatment, EDTA buffer or enzyme based antigen retrieval methods will be tried. The number of test rounds for each antibody is restricted to maximum three. Each antibody

approved by HPA will subsequently be used for immunostaining of eight TMAs representing 576 tissue spots.

Flow of information

The antibody provider (academic or commercial) will obtain the results of the quality assurance. Confirmation of successful tissue profiling (including annotation) will be sent to the collaborator together with an instruction (password to the internal web database) on how to access the results from the protein profiling. Information about non-approved antibodies will only be communicated to the antibody provider (and will not be published).

For academic collaborators, the data will be stored at least six months on the internal web atlas before transfer to the publicly available Protein Atlas (www.proteinatlas.org). Each approved antibody on the Protein Atlas will be directly linked to the collaborators web-home page.

Quality assurance of the antibody

A documentation of the validation should be submitted preferably through a web-link (product data sheet and/or reference) or alternatively as a pdf-file (with results, reference and/or product data sheet).

Antibodies should be validated by at least one of the following two methods:

1. Western blot (with relevant human tissue extracts and /or cells)
2. Immunohistochemistry (IHC with relevant human tissue and/or cells)

Examples of other suitable validation assays are:

- Antigen-based assays (ELISA, protein arrays or similar protein assay)
- Adsorption test (in combination with IHC)
- Flow sorting
- Immunocapture results (“pull-down” followed by suitable analysis)

Information about the antigen

Information about the antigen is also requested in the submission form. It is preferable (but optional) to give the size of the antigen in number of amino acids. In the comment field one can also add actual peptide sequence and information about purity check, sequence verification etc (optional).

Throughput and success rates

We foresee that approximately 800 to 1000 external antibodies can be pursued annually.

Priority

Not more than two antibodies to the same target protein will normally be pursued. Priority will thus be given to new targets not present (or only represented by one antibody) in the Protein Atlas or in the pipeline. Priority will be given to antibodies with well-documented and relevant quality assurance (see above).

Cost

The cost for the profiling (Western blot analysis, immunohistochemistry, scanning and annotation) will (during the pilot project) be covered by the HPA project (Wallenberg Foundation).

Release of data

Academic collaborators, can ask that the release of the data on the public Protein Atlas should be delayed further (beyond six months) if publication(s) based on the results from the protein profiling are being prepared. In this case, the release of the images can be delayed (pending decision by the HPA priority committee) normally not longer than one year.

Time frames and rules for the collaboration

1. If approved, confirmation of antibodies to be included in the HPA pipeline will be sent to the applicant (collaborator). This information will be kept confidential by the HPA. The collaborator is asked to send a vial of the approved antibodies to the HPA. A minimum amount of 50 µg or equivalent will be needed for each antibody aliquot, delivered in appropriate tubes (preferably 1-2 ml volume with screw cap) and properly labeled.
2. Each antibody is tested according to HPA-standard procedures, i.e., on Western blots and on a special designed TMA initially using two antibody-dilutions and heat epitope retrieval in citrate buffer.
3. The results of the antibody validation and the decision to approve or not approve the antibody for the Protein Atlas will be sent to the antibody provider. This information will also be kept confidential by the HPA.
4. Each antibody approved by HPA will subsequently be immunostained on eight TMAs representing 576 tissues and manually annotated.
5. Confirmation of successful tissue profiling (including annotation) will be sent to the collaborator together with instruction (password to the internal web database) how to access the annotated tissue array images with the results from the antibody. The collaborator is free to use this information for his/her own research or marketing (in the case of commercial vendors).