Frequently Asked Questions

Flash Oxidation (Fox™) Protein Footprinting Services for Higher Order Structural (HOS) Analysis

Easily Explore the Advantages of HRPF Via Fox Services

If you are working in biopharmaceutical and biosimilar development, or in protein conformational research, you can explore the benefits of GenNext's patented and novel Fox technology through contract services. This streamlined services program is the fastest and most economical route to access this robust and state-of-the-art HOS analytical method.

What is HRPF?

Hydroxyl Radical Protein Footprinting is a powerful method to study a protein's conformation by oxidatively modifying its solvent addressable surface, which in turn provides insight towards HOS.

Hydroxyl radicals, which oxidize the protein on the microsecond timescale, are generated by a proprietary high energy plasma flash oxidation system that was pioneered by GenNext.

To quantify the extent of oxidation, HRPF is coupled with LC-MS/MS to generate rich data quantifying labeling at different parts of the protein structure.

Then deep data analysis is performed using FoxWare™ Data Processing Software. The qualitative and quantitative insights generated by FoxWare Software inform and address key requirements in biopharmaceutical and biosimilar research.

Prior to this, HRPF required the use of prohibitively expensive and dangerous methods that are not practical in most laboratories.

What can HRPF be used for?

HRPF is proven to be particularly powerful for identifying protein interactions and changes in protein HOS. Key applications include ligand binding site identification, epitope mapping, biosimilar analysis, expression platform optimization and formulation strategies. Major applications for HOS using HRPF studies include:

- Antibody-antigen epitope and paratope mapping
- Protein-ligand interactions
- Protein-protein interactions
- Receptor-drug interactions

What new information will I receive beyond what can be obtained using traditional HOS methods?

If you are already working with traditional HOS methods, using GenNext's service will deliver novel information regarding the 3D structure of your molecule.

For example, HRPF can oxidize 19 out of 20 amino acid side chains—as opposed to Hydrogen Deuterium Exchange (HDX), which only indicates changes to backbone amide groups— and therefore provides richer solvent accessibility information.

Also, due to HRPF's irreversible label, the post-labeling workflow is flexible and can be customized for your specific study. For example, glycans can be easily removed post-labeling allowing the study of highly glycosylated proteins. Also, a wide range of proteolytic enzymes and long LC gradients can be used to ensure proper protein coverage.

Since HRPF products are stable, it is highly controlled and reproducible, delivering detailed structural information for greater confidence in your research results.

How does the service program work?

Each HRPF research program is tailored to meet your specific HOS analytical needs with an affordable fee-for-service approach.

Using a research collaboration model, together we will define a specific multi-phase approach that facilitates timely return of research results. Regularly scheduled meetings enable collaborative review of experimental details and program refinement.

All interactions are held under the strictest confidentiality to ensure protection of your company's proprietary data and intellectual property rights.

To start, you will be asked to provide the samples of your protein to be studied, along with appropriate ligands and buffers.

Next, GenNext's applied research scientists will perform the HRPF experiment using the Fox System in its laboratory near San Francisco, California. Following enzymatic digestion to constituent peptides, GenNext researchers will analyze the samples using LC-MS/MS.

Finally, GenNext experts will analyze the MS data using FoxWare™ Software and provide a comprehensive report on the HOS data and insights generated by protein footprinting.

How much sample do I need to use this service?

A full HRPF method development and differential study of the protein in two conditions requires at least 225 µL of protein at 0.8 mg/mL or 20 µM (whichever is higher). For each additional condition, an extra 75 µL of protein is required.

An epitope mapping study requires a minimum of 75 µL of antibody at the proper concentration to bind the antigen 1:1. A protein-ligand interaction study requires enough ligand so that, at equilibrium, 40-100% of the protein is bound.

For additional information, including buffer and vehicle requirements, please contact us at info@gnxtech.com.

What steps do I need to take to learn more about the service?

If you are interested in exploring Fox Services, email us at info@gnxtech.com. You will then be contacted by a member of our technical staff who will arrange a meeting to discuss the details of the project. Then, if you are interested in proceeding, GenNext will issue a quotation for the services that includes an estimated cost and timeline to complete the work.

Is it possible to purchase a Fox™ System for our laboratory?

Yes, GenNext offers its instrument and software for sale under normal commercial terms, and they can be found at leading biopharmaceutical firms, universities, and government labs.

Our Fox Services offers scientists who are new to HRPF a way to easily, quickly, and cost-effectively take advantage of the technology without the commitment of a capital equipment purchase. Services have also been used as a proof of principle before a system purchase.

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Discover the Benefits of Protein Footprinting

Contact us for products and services to investigate biopharmaceutical structure, interactions, folding, aggregation, formulation, and delivery.

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