

Here, all the basic information of your HDX-MS experiment needs to be uploaded.

Kingfisher HDX can read HDExaminer output files directly. Open HDExaminer, go to "Tools", select "Export," and click "Peptide Pool Results". You can now upload that .csv file directly to Kingfisher HDX.

If you are not using HDExaminer, you can still use Kingfisher HDX if you format your output data as seen in the test file (download the file using the bottom right link). Once you have formatted your data in the same way, upload your file and mark the checkbox for other output files.

In this tab you also need to input the FASTA file for your protein, number of protein states in your HDX experiment, the number of replicates per labeling time, number of labeling times, the significance level you want to use for the statistical analysis (we recommend using $\alpha=0.01$), the labeling times in seconds and the number of residues in the N-terminal tail of each peptide that cannot withhold deuteration (we recommend using 2).

The screenshot shows a web form titled "Data import and experiment details". It contains several input fields and checkboxes. Annotations with orange arrows point to specific elements:

- An arrow points to the "Select exported CSV file from HDExaminer" section, specifically to the "Browse..." button. A text box says: "Upload your HDExaminer output file here."
- An arrow points to the checkbox "Check if you are NOT using an HDExaminer output file". A text box says: "Mark this checkbox if you are NOT using an HDExaminer output file."
- An arrow points to the "Select FASTA file" section, specifically to the "Browse..." button. A text box says: "Upload your protein FASTA file here."
- An arrow points to a link at the bottom right that says "Download a test file here". A text box says: "Download the .csv test file here."

The form fields include:

- "Select exported CSV file from HDExaminer": "Browse..." button, "No file selected" text.
- Checkbox: "Check if you are NOT using an HDExaminer output file".
- "Select FASTA file": "Browse..." button, "No file selected" text.
- "Number of protein states": Dropdown menu with value 2.
- "Number of labeling times": Dropdown menu with value 4.
- "Significance level": Dropdown menu with value 0.01.
- "Number of replicates per labeling time": Dropdown menu with value 4.
- "Number of residues that can't withhold deuteration": Radio buttons for 1 and 2 (2 is selected).
- "Labeling times in seconds (separated by commas)": Text input field with value "30,60,800,2500".
- Bottom right link: "Download a test file [here](#)".

Once you have uploaded both your .csv output file and FASTA file, a new button will appear below the black line. Click "calculate" to start the analysis.

If the analysis is successful, two dropdown lists will appear below. One to select your reference state and the other to select the protein state that you want to compare it with.

Once you selected your state 1 (reference) and state 2, you can now go navigate to all other tabs in the left menu.

Note: If when clicking "calculate" you get an error message, please check all your input data carefully and reload the webpage. Please remember that if you are not using an HDExaminer output file you will need to manually format your data as seen in the .csv test file.

Data import and experiment details

Select exported CSV file from HDEaminer

Browse... HDX export file test.csv

Upload complete

☐ Check if you are NOT using an HDEaminer output file [i](#)

Select FASTA file

Browse... NIST mAb HC.fasta

Upload complete

Number of protein states

2

Number of replicates per labeling time

4

Number of residues that can't withhold deuteration:

- ☐ 1
- ☒ 2

Labeling times in seconds (separated by commas):

30,60,800,2500

Calculate

Number of labeling times

4

Significance level

0.01

The calculate button will only appear once you have uploaded your files.

Select reference state:

NIST mAb pH 6

Select state 2:

NIST mAb pH 5

Select your reference state and the protein state you want to compare it with.

[Download a test file here](#)

If you are NOT using an HDEaminer output file, please format your data as follows (must be a .csv file):

1	State	pepnumber	Start	End	Sequence	Charge	30s_1	30s_2	30s_3	30s_4	60s_1	60s_2	60s_3	60s_4	800s_1	800s_2	800s_3	800s_4	2500s_1	2500s_2	2500s_3	25
2	NIST mAb pH 6	5	11	RESGPAL	1	3.5	3.5	3.5	3.5	3.5	-1.8	-1.8	-1.8	-1.8	2.359	2.183	2.051	2.311	2.056	2.322	2.457	
3	NIST mAb pH 6	5	19	RESGPALVKF	3	3.463	3.458	3.405	3.415	4.028	4.102	4.141	4.028	4.866	4.908	4.813	4.867	5.167	5.28	5.252		
4	NIST mAb pH 6	5	20	RESGPALVKF	3	3.454	3.215	3.314	3.362	3.896	3.915	3.966	3.99	4.452	4.427	4.483	4.264	4.945	4.682	4.749		
5	NIST mAb pH 6	21	26	TCTFSG	1	NA	NA	NA	NA	1.597	NA	NA	NA	1.417	1.846	1.869	NA	NA	NA	NA		
6	NIST mAb pH 6	27	50	FSLSTAGMSI	3	4.604	4.726	4.631	4.449	5.184	5.229	5.329	5.31	5.641	5.697	5.658	5.702	6.029	6.088	6.16		
7	NIST mAb pH 6	28	50	SLSTAGMSVI	3	4.051	3.861	3.946	3.79	4.304	4.43	4.505	4.576	5.112	5.045	5.033	5.049	5.229	5.343	5.301		
8	NIST mAb pH 6	35	50	SVGWIRQPP	3	2.299	2.225	2.208	2.22	2.65	2.721	2.756	2.711	3.039	3.023	3.035	3.053	3.138	3.142	3.146		
9	NIST mAb pH 6	35	52	SVGWIRQPP	3	2.596	2.463	2.333	2.557	2.975	3.127	3.143	3.046	2.878	3.15	3.201	3.028	3.183	2.85	3.077		
10	NIST mAb pH 6	36	47	VGWIRQPPG	3	2.323	2.013	1.966	1.891	2.006	2.153	2.26	2.227	2.305	2.558	2.061	2.283	2.354	2.23	2.562		
11	NIST mAb pH 6	36	48	VGWIRQPPG	3	2.207	2.045	2.152	2.002	2.204	2.332	2.34	2.173	2.213	2.341	2.163	2.3	2.204	2.187	2.445		
12	NIST mAb pH 6	36	50	VGWIRQPPG	3	2.153	2.165	2.121	2.144	2.524	2.551	2.448	2.629	2.892	2.953	2.833	2.88	2.975	3.007	3.079		
13	NIST mAb pH 6	38	50	WIRQPPGKA	3	2.006	1.955	2.035	2.07	2.361	2.328	2.7	2.321	2.542	2.564	2.563	2.77	2.73	2.913	2.516		
14	NIST mAb pH 6	39	50	IRQPPGKALE	3	2.538	2.444	2.427	2.249	2.718	2.737	2.915	2.783	2.845	3.1	2.952	3.247	3.28	3.077	2.866		
15	NIST mAb pH 6	47	50	LEWL	1	0.446	0.295	0.446	0.287	0.523	0.529	0.654	0.584	0.859	0.863	0.835	0.911	0.914	0.926	0.931		
16	NIST mAb pH 6	47	54	LEWLADIW	1	1.519	1.49	1.402	1.36	1.65	1.709	1.701	1.722	2.335	2.348	2.33	2.355	2.944	2.986	2.967		
17	NIST mAb pH 6	49	52	WLAD	1	0.286	0.273	0.257	0.264	0.244	0.26	0.31	0.277	0.218	0.265	0.298	0.269	0.271	0.174	0.27		
18	NIST mAb pH 6	49	65	WLADIWWD	4	1.986	1.851	1.952	1.834	2.1	1.927	2.162	2.048	2.394	2.491	2.251	2.402	2.477	2.676	2.73		
19	NIST mAb pH 6	49	69	WLADIWWD	5	2.442	2.035	2.151	2.086	2.789	3.232	3.036	2.789	3.734	3.795	3.783	3.824	4.546	4.661	4.48		
20	NIST mAb pH 6	51	65	ADIWDDKKI	4	2.054	1.963	1.831	1.802	2.318	2.24	2.268	2.112	2.446	2.595	2.212	2.334	2.56	2.752	2.687		
21	NIST mAb pH 6	51	69	ADIWDDKKI	4	2.551	2.378	2.392	2.165	3.495	3.284	3.229	2.899	3.954	4.014	3.899	3.825	5.201	4.858	4.946		
22	NIST mAb pH 6	53	65	IWWDDKKHY	3	2.286	2.391	2.388	2.265	2.371	2.37	2.549	2.52	2.902	2.791	2.53	2.503	3.053	2.925	3.096		
23	NIST mAb pH 6	53	69	IWWDDKKHY	4	2.62	2.263	2.373	2.368	2.866	2.855	2.989	2.922	3.792	3.955	3.868	3.803	4.885	4.827	4.656		