

# EpiQuest-H – Operation Manual



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## What is EpiQuest-H?

EpiQuest-H allows you to build the hydrophobicity profile (and to determine the hydrophobic or hydrophilic regions) by simultaneously using matrixes based on several different hydrophobicity profiles proposed for amino acids. All different profiles were used to generate Matrices with a comparable spread of positive and negative values, which makes the resulting values also more comparable.

## Choosing Algorithms

Algorithms to include	
<input checked="" type="checkbox"/> Kyte & Doolittle (HKD2)	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR
<input checked="" type="checkbox"/> HWW2	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR
<input checked="" type="checkbox"/> Bull & Breese (HBB2)	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR
<input checked="" type="checkbox"/> Zhu et al (HZZ2)	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR
<input checked="" type="checkbox"/> Hopp & Woods (HHW2)	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR
<input checked="" type="checkbox"/> Eisenberg (HEW2)	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR
<input checked="" type="checkbox"/> Zhao & London (HZZ2)	<input type="checkbox"/> GRAPH <input checked="" type="checkbox"/> BAR

You can choose several different algorithms, based on the respective matrices proposed by every group<sup>1</sup>. The most standard one is after **Kyte & Doolittle**. You may also choose how you would like the results to be displayed: as Graphs or Bars (the latter only shows the positive regions).

## Entering the Data

To analyse the sequence, please simply Paste it into the window for Sequence. The sequence must be in a single-letter format.

**Protein Name**

**Accession Number**

**Sequence in FASTA**

Filter

Filter

Start:  End:

We recommend also entering the accession number of the sequence you use and the name of your sequence or project. This data will be present in all **Results** you save after the analysis.

If your sequence contains gaps (may appear due to formatting) or contains abnormal signs (punctuation/letters that are not amino acid code) the analysis will be aborted. To avoid it, keep the "Filter" option selected.

To analyse only a fragment of the sequence, please enter the position number of the first and the last amino acids of the fragment you wish to analyse. Please be aware that the numeration of the sequence starts from the first amino acid of the chosen sequence and will be kept during the analysis of the fragment. I.e. if the first aa of the fragment was 101, it will be indicated as 101 in all further analysis and Results.

However, if you paste into sequence window a fragment of some sequence, its first amino acid will be referred as in position 1, not the position in the original sequence- - source of the fragment.

## Settings for Accessibility analysis

### Settings for Analysis

Frame size:  ∈ [1, 31]  
Gap size:  ∈ [0, 10]  
Threshold:  ∈ [-3, 3]  
Peptide size:   higher  
Sort by:

You can use the Default values, but also, depending on the requirements of your analysis, vary some of the settings.

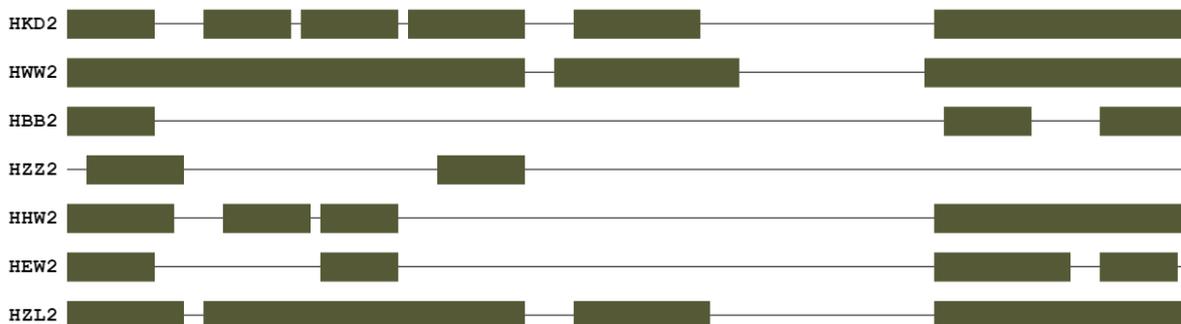
Your input may be reset back to the **Default** settings by pressing the respective button.

**Frame size** defines the size of the context sequence that will be reviewed for every position. We recommend setting the value to 6, but if you want to map the Hydrophobicity more precisely, you can use a lower setting (down to 2). When you use low settings for Frame size you will end up with many

shorter hydrophilic fragments in your Report; usually such fragments are interrupted by one/two hydrophobic acids. You may join them by allowing the presence of hydrophobic residues among hydrophilic fragments, by increasing **Gap size**. Gap size indicates the length of hydrophobic inserts allowed between hydrophilic fragments for them to be viewed as one.

Normally, your results will be reported as uninterrupted positive sequences of length equal to or exceeding the length defined in the **Peptide Size** setting. Sometimes small gaps may interrupt the otherwise long fragment. This may be a result of occasional enrichment of the region with amino acids of a certain type but does not mean that the sequence is inaccessible. To avoid such interruption, you may use the settings for Gap Size from 1 to 3, in rare situations up to 5. The recommended default value is 1.

**Threshold (Th)** : you may want to discriminate only the peaks from the rest of data, in this case rise



the Th to 1 or even 3.

Threshold =0

Threshold =2



**Peptide size:** while the Graph in Results represents the values for every amino acid of the sequence with the peak positive and negative values, the Bar and Tabular reports show only **positive fragments** that are equal to or above the length of a sequence defined in Peptide Size. For that, the **“and higher”** option should be selected. If it is not selected, the Table will show all positive peptides of the chosen parameters (i.e. 8-mers, overlapping, with at least one aa difference).

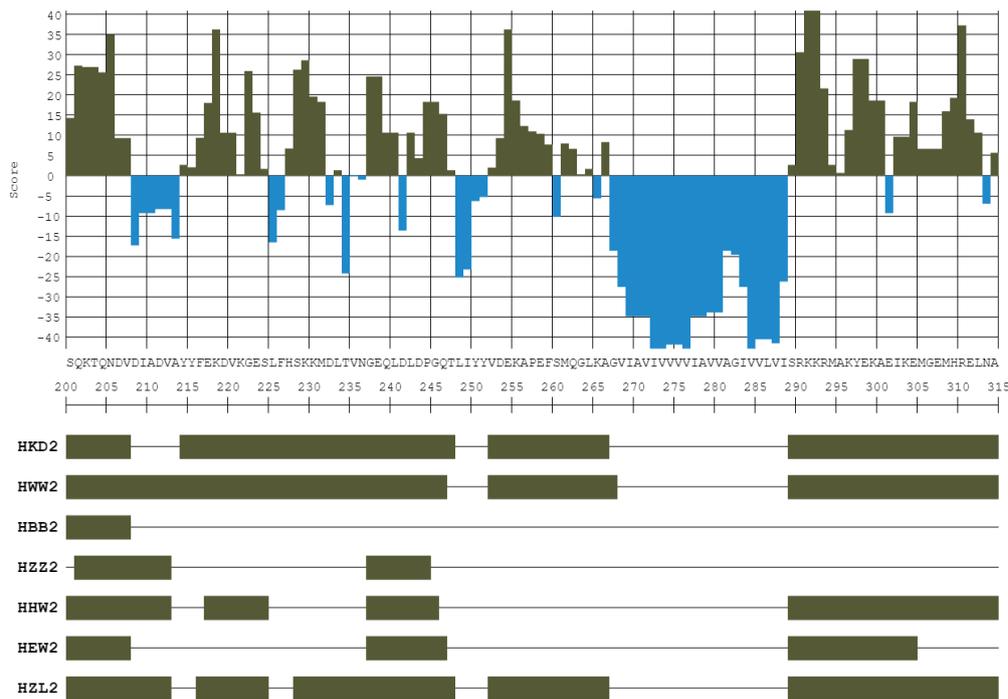
**Sort by** defines how the results for positive fragments will be presented in the report tables: sorted according to the order of the fragments in the original sequence (*Start*), according to their *Length* (longest goes first), cumulative antigenicity of the fragment *CH* (cumulative hydrophilicity) or CH per amino-acid residue of the fragment (*CHR*).

**Chart Type:** The Chart for analysis will be presented as a **Graph** or just **Bar** indicating only the positions of positive sequences with length equal or above the selected in **Peptide Size**. In the stand-alone program both types of graphical output are presented in the results.

## Viewing and saving the Results

The results of the analysis are presented in 2 ways, graphical and tabular.

Epithelial cell adhesion molecule (EPCAM); P16422.2  
Detailed view for Hydrophobicity: (Matrix HKD2, F02T00G02)



The Graphical output contains the name of the sequence (name of the project), accessory number of the sequence, Matrix used, and the parameters you have chosen for the analysis, where F stands for Frame, T for Threshold and G for the sizes of allowed gaps (in the image shown F=6, T=0, G=0: F06T00G00)

You may save the entire diagram by clicking the Save diagram button above it. The image will be saved in a PNG format with a resolution of 96 ppi.

The **Tables** of results show the sequences for the detected antigenic fragments. They will be reported in order of parameters chosen in Settings.

[View](#) [Save](#) [Copy](#) **Report for "HKD2":**

Matrix = HKD2 / F = 2 / G = 2 / T = 0 / Peptide size = 8+

Start	End	Length	CH	CHR	Sequence
200	207	8	174.88	21.86	SQKTQNDV
214	247	34	301.97	8.88	YYFEKDVKGESLFHSHKMDLTVNGEQLDLDPGQT
252	266	15	116.84	7.79	VDEKAPEFSMQGLKA
265	288	24	-757.39	-31.56	KAGVIAVIVVVVIAVVAGIVVLVI
289	314	26	391.12	15.04	SRKKRMAKYEKAEIKEMGEMHRELNA

[View](#) [Save](#) [Copy](#) **Report for "HWW2":**

Matrix = HWW2 / F = 2 / G = 2 / T = 0 / Peptide size = 8+

Start	End	Length	CH	CHR	Sequence
200	246	47	790.54	16.82	SQKTQNDVDIADVAYFEKDVKGESLFHSHKMDLTVNGEQLDLDPGQ
252	267	16	245.41	15.34	VDEKAPEFSMQGLKAG
268	288	21	-186.83	-8.90	VIAVIVVVVIAVVAGIVVLVI
289	314	26	529.81	20.38	SRKKRMAKYEKAEIKEMGEMHRELNA

[View](#) [Save](#) [Copy](#) **Report for "HBB2":**

Matrix = HBB2 / F = 2 / G = 2 / T = 0 / Peptide size = 8+

Start	End	Length	CH	CHR	Sequence
200	207	8	187.80	23.48	SQKTQNDV
208	217	10	-184.50	-18.45	DIADVAYFE
264	289	26	-582.75	-22.41	LKAGVIAVIVVVVIAVVAGIVVLVIS
296	303	8	8.85	1.11	KYEKAEIK

[View](#) [Save](#) [Copy](#) **Report for "HZZ2":**

Matrix = HZZ2 / F = 2 / G = 2 / T = 0 / Peptide size = 8+

Start	End	Length	CH	CHR	Sequence
201	212	12	120.90	10.08	QKTQNDVDIADV
221	230	10	-43.76	-4.38	KGESLFHSHK
227	244	18	80.78	11.16	GEQIDIDP

It contains the settings of the analysis, position of the fragment (Start-End), its length, cumulative Surface Exposure index (SEPI) and an index per amino-acid (SEPR).

You may **Copy** the Table and just Paste it into an Excel sheet or Text (Word, .RTF etc).

You may **View** the Report in complete form, with the exact analysed sequence and print it into PDF using your browser Print function or **Save** it for your records as a standalone HTML document that may be further converted into other formats.

## Demo Sequences

For Demo and training purposes, we supply several sequences with known with known accessible and inaccessible areas, including transmembrane domains and areas mapped by antibodies to various linear epitopes.

You may choose them by opening the drop-down list **-Select a demo sequence-**.

Clear ---Select a demo sequence---

---Select a demo sequence---

- Epithelial cell adhesion molecule (EPCAM); P16422.2
- Full=Alpha-2B adrenergic receptor; P18089.4
- Tetraspanin-18 [Homo sapiens]; NP\_570139.3
- Podoplanin [Homo sapiens]; Q86YL7.3

Filter Start: 200 End: 314

[In our blog](#) we further show how to use the program in combination with other ones to address different research tasks.

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<sup>i</sup> [References to original hydrophobicity matrixes](#)