

# Fragment Screening by WAC™

**WAC™ (weak affinity chromatography) is a new technology for fragment screening which enables fragment screening using standard HPLC-UV/MS equipment. WAC™ represents an affordable, accessible and reliable alternative or complement to existing techniques for fragment screening. Large fragment libraries can be screened with high throughput by running them in mixtures and using mass spectrometry detection.**

## How does it work?

WAC™ is based on HPLC where the target protein is immobilized onto the support material of a HPLC column. In WAC™, retention of fragments on the column is related to selective interaction of the fragment with the immobilized target protein. A physiological buffer is used as mobile phase.

WAC™ has proven as a reliable method by comparison with existing techniques for fragment screening, such as NMR, SPR, thermal shift assays and x-ray crystallography(1).

## How do I use WAC™?

Fragment screening by WAC™ is a relatively straight forward process. The target protein is immobilized by covalent amine coupling onto a WAC™ column. Lysine side chains on the protein surface are employed for stable covalent immobilization.

The amount of active protein present on the immobilized WAC™ column ( $B_{tot}$ ) can be determined by frontal affinity chromatography (FAC), or estimated by analysis of a reference compound of known affinity. This information is required to determine affinities of fragments during screening.

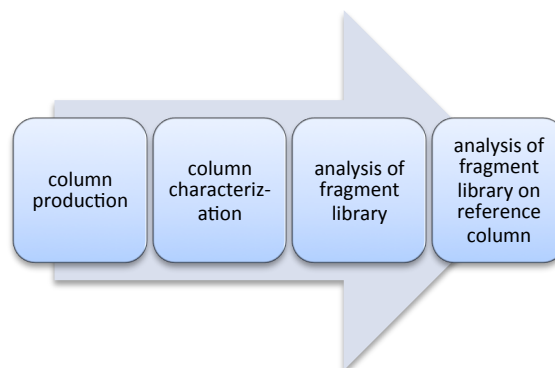


Figure 2. The process of fragment screening by WAC™.

In order to distinguish selective binding of a fragment to the target protein/the active site of the protein, screening on a reference column is required. This can either be a blank column with no immobilized target protein, or a column with immobilized target protein where the active site has been blocked by either a covalent binder or a high affinity non-covalent binder present in the mobile phase.

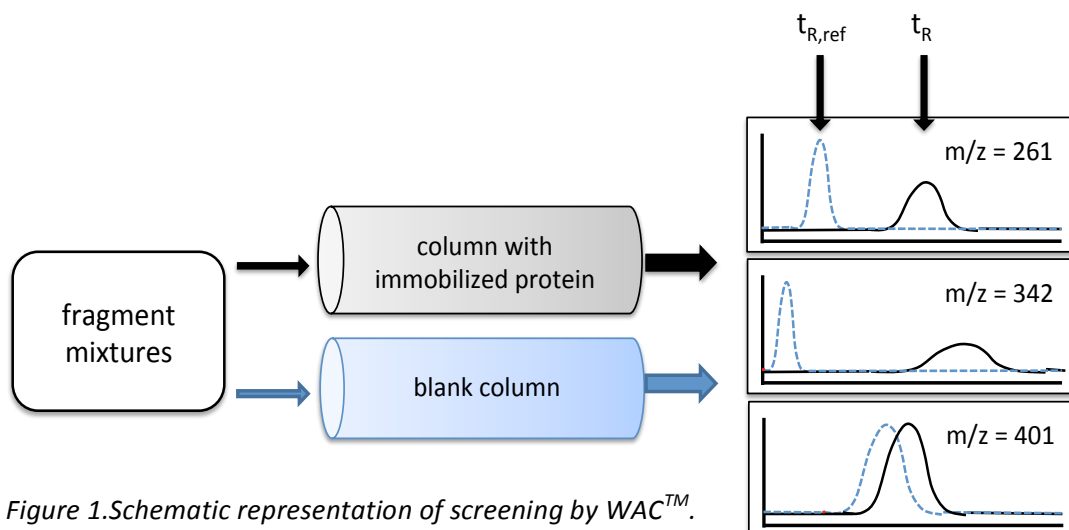


Figure 1. Schematic representation of screening by WAC™.



Weak Affinity Chromatography

The affinity ( $K_D$ ) of the fragment to the target protein is determined from the retention time on the column ( $t_R$ ) by a single injection – it is not required to set up a binding isotherm. The  $K_D$  is determined by the following expression:

$$K_D = \frac{B_{tot}}{F \cdot (t_R - t_{R,ref})}$$

where  $F$  is the flow rate,  $B_{tot}$  the amount of active protein on the column,  $t_R$  the retention time of the fragment on the WAC™ column and  $t_{R,ref}$  the retention time on the reference column.

A WAC™ column is usually very stable as the immobilized protein is significantly more stable than when present in solution. Proteins such as thrombin and Hsp90 has proven to maintain 90% activity for several months and >200 injections(1,2). The activity of the target protein can be continuously monitored by including a reference compound in each fragment mixture during screening.

#### Benefits of fragment screening by WAC:

- Fragment screening can be performed on standard HPLC equipment
- affordable
- relatively high throughput
- reliable
- robust
- affinity determination possible by a single injection
- very low fragment consumption
- chiral analysis possible(4,5)
- enables analysis of complex matrices and crude reaction mixtures(6)

#### What do I need?

- a standard HPLC instrument
- a MS detector for high throughput screening by fragment screening in mixtures where each individual fragment is detected by its  $m/z$
- a WAC™ column with immobilized target protein

#### Overview

<b>Affinity range of fragments</b>	~2 mM – 1 $\mu$ M
<b>Sample consumption</b>	0.04 nmol/injection
<b>Protein consumption (capillary LC system)</b>	1-5 mg (100-200 $\mu$ g)
<b>WAC™ column stability</b>	$\geq$ 200 injections(1-2)
<b>throughput</b>	3600 fragments/24 h(3)

#### Transientic Interactions offers:

- WAC™ columns for protein immobilization
- WAC™ columns with your immobilized target protein of interest
- Consulting services
  - screening of your fragment library by WAC™
  - mixture design
  - column characterization
  - professional training

#### For ordering information etc.:

contact@transientic.com

#### References

1. Meiby E, Simmonite H, le Strat L, Davis B, Matassova N, Moore J, et al. Fragment screening by weak affinity chromatography (WAC): comparison with established techniques for screening against HSP90. *Anal Chem.* 2013;14(85):6756–66.
2. Duong-Thi M-D, Meiby E, Bergström M, Fex T, Isaksson R, Ohlson S. Weak affinity chromatography as a new approach for fragment screening in drug discovery. *Anal Biochem.* 2011;414(1):138–46.
3. Duong-Thi M-D, Bergström M, Fex T, Isaksson R, Ohlson S. High-throughput fragment screening by affinity LC-MS. *J Biomol Screen.* 2013;18(2):160–71.

4. Duong-Thi M-D, Bergström M, Fex T, Svensson S, Ohlson S, Isaksson R. Weak affinity chromatography for evaluation of stereoisomers in early drug discovery. *J Biomol Screen.* 2013;18(6):748–55.
5. Meiby E, Knapp S, Elkins J, Ohlson S. Fragment screening of cyclin G-associated kinase by weak affinity chromatography. *Anal Bioanal Chem.* 2012;404(8):2417–25.
6. Meiby E. Progress of Weak Affinity Chromatography as a Tool in Drug Development. Linnaeus University; 2013.