ASSAY DEFENDER®

Total HAMA and interference blocker

Ready-to use assay diluent for reduction of interference caused by HAMA (human anti-mouse antibodies), rheumatoid factors, nonspecific binding, cross-reactivities and matrix effects.

Interference in immunoassays can cause false positive and false negative results in routine testing. Reasons for the increasing prevalence of different kinds of interference are for example:

- population aging
- a growing number of patients with:
  - chronic autoimmune diseases
  - allergic and inflammatory diseases
- a better limit of detection in modern assays
- more applications of sandwich-assay formats

Interferences can be classified due to their biochemical reasons and impact on assay performance.

1 **Interference caused by antibodies from patient samples**
e.g. HAMA (human anti-mouse antibodies), HAAA (human anti-animal antibodies), heterophilic antibodies and rheumatoid factors from patient samples

2 **Interference caused by endogenous components of the sample**
e.g. albumins, complement, lysozyme, fibrinogen, α-1 Antitrypsin, atypically high lipid-, salt- or sugar concentrations as well as atypical viscosities

3 **Interference caused by assay components**
Assay components - like fluorescent or enzymatic labels - can cross-react with substances from the sample or change binding properties of the assay antibodies

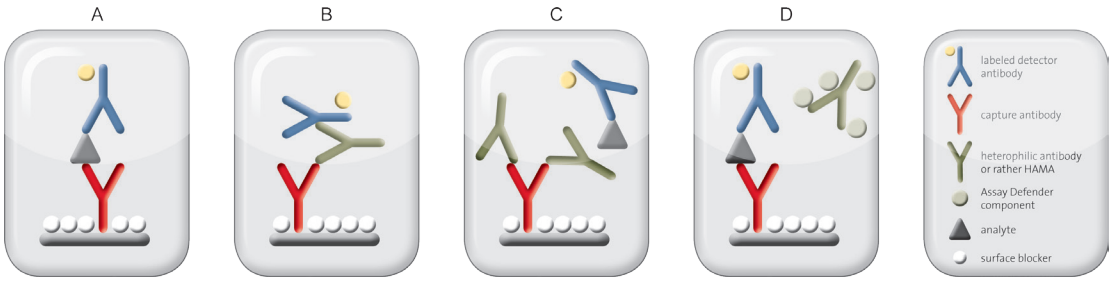


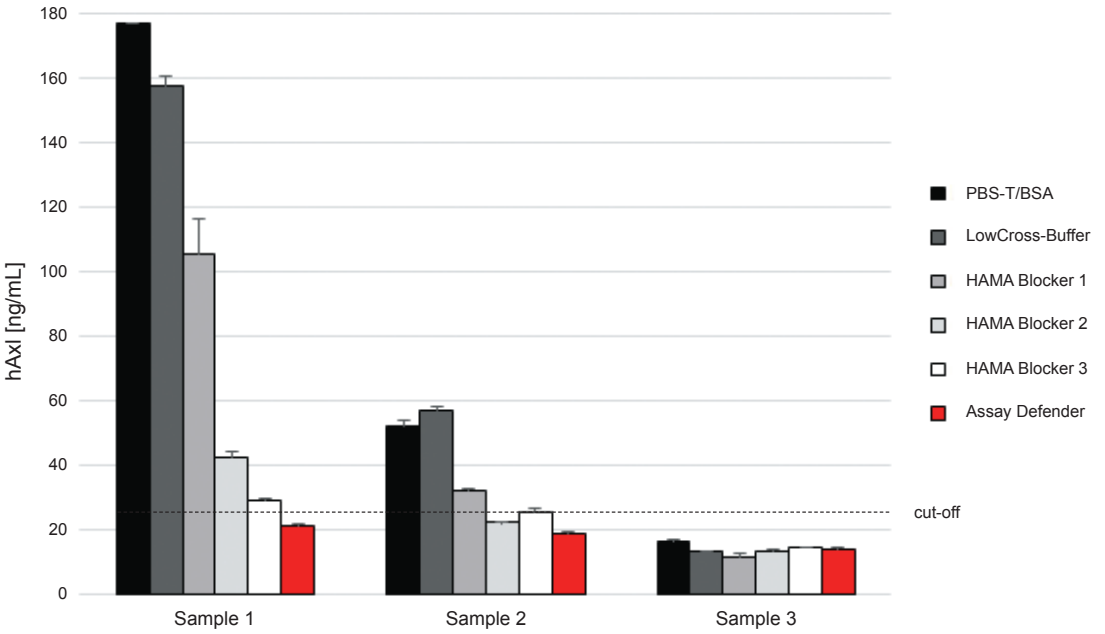
Fig. A: Normal sandwich assay with no interference → correct result
Fig. B: An interfering antibody forms a bridge between capture and detection antibody → false positive result
Fig. C: Binding of the interfering antibodies to the capture antibody → false negative result
Fig. D: Components of Assay Defender® prevent binding of the interfering antibody → correct result

Assay Defender® enables effective elimination of all kinds of interference and helps to prevent false positive or false negative results.

Use of Assay Defender®

Assay Defender® can be used for immunoassays using capture and/or detection antibodies. It is particularly suitable for human or animal specimens containing HAMA (human anti-mouse antibodies) or other AAA (anti-animal antibodies).

Assay Defender® is a ready-to-use dilution buffer for human or animal body fluid specimens. The previously used dilution buffer can be easily replaced by Assay Defender®. Calibrators must also be diluted in Assay Defender®. Dilution in Assay Defender® can be 1:2 or higher. A useful dilution factor for most applications is 1:10 (1 part specimen + 9 parts Assay Defender®).



Assay Defender® excels at preventing false positive results in interference samples

Serum samples from patients with rheumatoid illnesses (Sample 1 and 2) were analyzed using an established research ELISA for the detection of hAxl, a biomarker for hepatocellular carcinoma. Sample 3 is an interference-free sample from a healthy donor. Samples were diluted either in pure PBS-T/BSA, PBS-T/BSA supplemented with a commercially available HAMA Blocker (HAMA Blocker 3), commercially available ready-to-use HAMA blocking diluents (HAMA Blocker 1 and HAMA Blocker 2), or CANDOR's Assay Defender®. Assay Defender® alone can completely eliminate interferences and hence give a true negative result (< 25 ng/mL) in both interference samples. The results of interference free samples are not affected by Assay Defender®.

order numbers		
Assay Defender®	50 mL	180 050
Assay Defender®	125 mL	180 125
Assay Defender®	500 mL	180 500

Bulk quantities available on request for kit manufacturers

CANDOR®
The ELISA Experts

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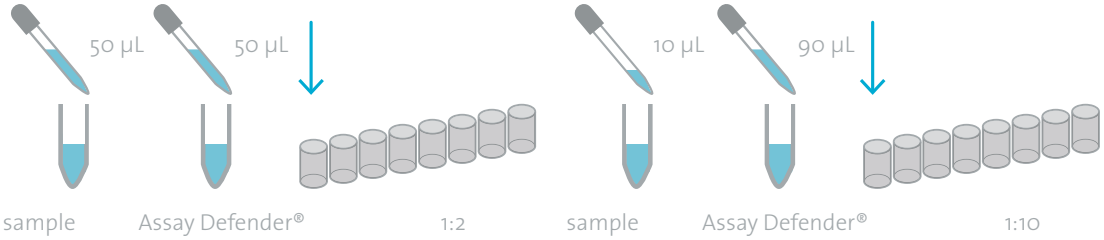
Examples for use of Assay Defender®

The use of Assay Defender® is always important when analyte and antibody meet and thus a high risk of interference effects is given.

ELISA

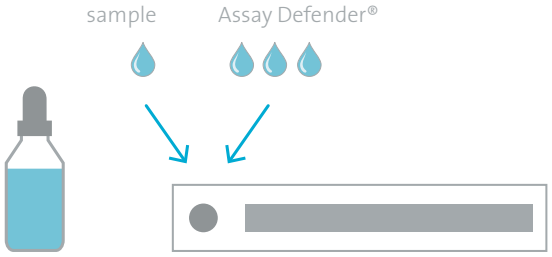
Assay Defender® replaces the ordinary sample diluent.
Specimens should be diluted with Assay Defender® at least 1:2, if possible 1:10 (1 part specimen + 9 parts Assay Defender®).



Higher dilutions are not a problem, provided the analyte concentration is high enough.
Assay Defender® is also applicable for immuno-PCR and protein arrays as sample diluent.

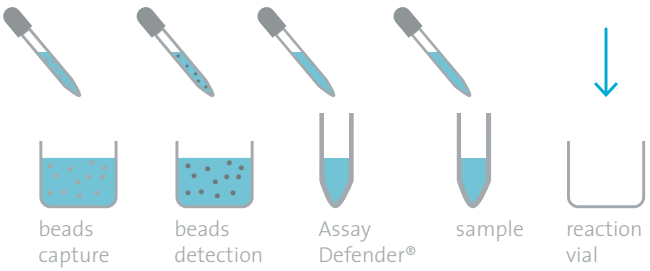
Lateral Flow Assay

Assay Defender® replaces flow buffer or chase buffer.



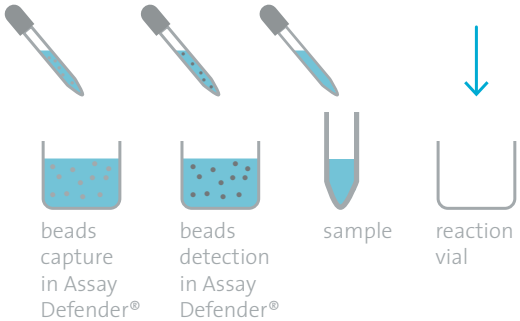
Bead-based platforms with sample diluent

Assay Defender® replaces the ordinary sample diluent. Dilution depends on the analyte concentration.



Bead-based platforms without diluent

Assay Defender® replaces the storage buffers for capture and detection beads.



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Assay Defender® *(article no. 180)*

Sample diluent for blocking HAMA and other high affinity interfering antibodies and minimizing nonspecific binding, cross-reactivities and matrix effects in immunoassays based on human or animal body fluids

Storage:	2 - 8 °C
pH-value:	7.2 ± 0.2
Preservative:	contains < 0.0014 % [w/w] reaction mass of CMIT/MIT (3:1)
Expiry date when stored unopened:	please refer to the label on the bottle

For research use only, not for diagnostic use

Fields of application:
Assay Defender® is used as a ready-to-use dilution buffer for human or animal body fluid specimens in sandwich or competitive assay formats. Application areas are different assay technologies, such as ELISA, protein arrays, bead assays (e.g. Luminex assays), immuno-PCR, automated high-throughput immunoassay systems or lateral flow assays (as chase or flow buffer). *Assay Defender*® prevents faulty results caused by nonspecific cross-linking of capture and detection antibody due to high affinity interfering antibodies like HAMA and interferences caused by nonspecific binding, cross-reactivities and matrix effects. Addition of other HAMA blockers to *Assay Defender*® is not necessary.

Instructions for use
Assay Defender® is ready-to-use. Please shake the buffer thoroughly before use.

Dilution of the specimens:
Standards and specimens can be diluted with *Assay Defender*® at 1:2 or higher. A useful dilution in *Assay Defender*® for most applications is 1:10 (1 part specimen in 9 parts *Assay Defender*®). Standards and specimens should be treated strictly the same way.

Note: *Assay Defender*® is not used as diluent for antibodies. We recommend using *HRP-Protector*™ (article number 222), *LowCross*® *HRP-Stab* (article number 270) or *AP-Protector*® (article number 235) for the dilution of detection antibodies.

Appearance of signal reduction:
In some cases, a smooth reduction of the wanted signal can be observed. *Assay Defender*® reduces low and medium affinity binding. That means that by using polyclonal antibodies (which also contain low and medium affinity binding components) a smooth reduction of signals can appear.
In this case a moderate increase of the concentration of the antibody can lead to the previously seen signals. Unwanted low and medium affinity binding will still be reduced by *Assay Defender*®.

By using monoclonal antibodies with low or medium affinity binding, complete reduction of signal can occur. We recommend using suitable antibodies with high affinity.

Suitability of *Assay Defender*® for a specific assay has to be tested by the user.

Even if *Assay Defender*® is used as an assay diluent, it is still necessary to saturate surfaces like ELISA-wells with a surface blocker. We recommend using *The Blocking Solution* (article number 110).

For further information please visit www.candor-bioscience.com.