



## Application Note

# ADSORPTION OF CAPTURE ANTIBODIES ON HYDROPHOBIC ABICAP® HP COLUMNS Sandwich enzyme immunoassay for the detection of Francisella Tularensis LPS

### Introduction

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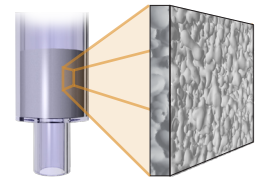
## Introduction

Sandwich enzyme immunoassays are used for the detection of antigens in various liquid matrices.

In this application note the technology for the production of a sandwich enzyme immunoassay on ABICAP-HP test columns is described using LPS from *F. tularensis* as model analyte. The approach is applicable for many other sandwich applications and comparable to standard ELISA technique. In the presented example PolyHRP in combination with precipitating TMB and photometric read out is described.

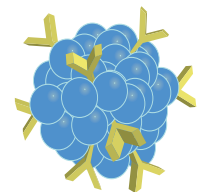
The advantage of the flow through ABICAP immunoassay technology is the possibility to enrich the analyte and to shorten the assay time considerably.

related document



[Introduction to  
3D Immunofiltration \(.pdf\)](#)

related document



[Introduction to  
PolyHRP technology \(.pdf\)](#)

## Materials

- senova abicap® HP columns
- senova abicap® Pipeting Rack
- ABICAP Reader (for direct read out)
- alternatively ELISA photometer (for read out after elution)
- 99% ethanol; 50% Ethanol
- carbonate buffer\*
- blocking buffer
- anti-FT monoclonal
- monoclonal anti-FT-biotin
- SA PolyHRP 40
- TMB-substrate
- sample dilution buffer\*
- washing buffer\*
- substrate buffer\*

\* see buffer recipe or senova pricelist

## Methods

### Adsorption of antibody to Senova Abicap HP column

In order to prepare the Senova Abicap HP column for adsorptive Protein binding, put your needed amount of ABICAP columns in Abicap frame and degas filter in Senova Abicap waste basket containing 130 ml ethanol at about 20 mbar for 10 minutes.

After degassing is completed (bubble formation has stopped and the filter shows an unruffled surface) the Abicap Frame has to be placed into Abicap Workstation (see fig. XXX)

#### ordering information

abicap® HP column	1.7.005.001
abicap® Pipetting Rack	1.7.005.001
abicap® Photometer	1.7.005.002
blocking buffer	1.7.005.003
anti-FT monoclonal	1.7.005.004
monoclonal aFT biotin	1.7.008.002
SA- PolyHRP	1.7.008.005
TMB substrate	5.1.001.002

#### buffer recipes

##### carbonate buffer

0,1 M NaHCO<sub>3</sub>; 0,1 M Na<sub>2</sub>CO<sub>3</sub>; pH 9,5;  
0,05% Microzide

##### sample dilutions buffer

1 part casein buffer concentrate  
(1.6.012.001); 3 parts 0,15M PBS pH  
7,3(0,05% Microzide)

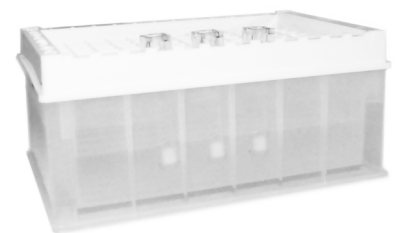
##### washing buffer

0,15M PBS pH 7,3 (0,05% Microzide);  
0,1% BSA; 0,05% Tween 20

##### substrate buffer

0,1M sodiumacetate; 0,1M citric acid;  
0,05% Microzide

fig 1: Abicap Rack in ethanol



In the next step the ethanol is replaced by carbonate buffer.

To this end the following liquids are added in sequential mode:

- 50% ethanol/ water 750µl
- Water 750µl
- 2\* Carbonate buffer 750µl

For adsorption of antibody add 7,5µg anti-FT in 750µl carbonate buffer and incubate 20 minutes at roomtemperature.

**Remark:** Typically 1-15µg protein in 250-750µl are applied per filter. The incubation time can be varied between 10-16 minutes. Cover the columns (eg. Parafilm) to prevent the filter surface gets contaminated

Finally the filter should be blocked to avoid unspecific binding. Add 750µl Senova blocking buffer to the columns and incubate the covered columns for 20 minutes.

**Remark:** To assure blocking, repeat these step if applicable one more time. Other blocking formulation can be used.

### Detection of antigen on coated Filter

To capture the analyte on the filter 750µl of sample solution are added (flow through time 2 minutes) followed by an additional incubation time of 4 minutes.

**Remark:** The recommended incubation time of 6 minutes is typical for most analytes. In some cases the kinetics may be different and incubation time needs to be adopted respectively

fig 1: Abicap Rack under vacuum



#### keynotes: adsorption on filter

- Degas abicap HP columns in ethanol
- 50% ethanol/ water 750µl (2 min)
- Water 750µl (2 min)
- Carbonate buffer 750µl (2 min)
- Add antibody in carbonate buffer, typically 1-15 µg antibody in 250 to 750µl liquid, Incubate 45 min
- Add protein Containing blocking buffer, incubate 10 min

#### keynotes: detection of antigen

- Add 750 µl analyte solution (6 min)
- Add 750 µl biotinate conjugate (6 min)
- Add 750 µl poly HRP-SA (6 min)
- Add 3 times 750 ul washing buffer
- Add 500 ul TMB substrate solution,
- wait 6 minutes
- Stop enzyme reaction by adding 750µl washing solution

Sandwich complex is formed by adding 750µl anti-FT biotin-conjugate (1µg/ml XXX) and additional 4 minutes incubation time.

For detection of the complex 750µl PolyHRP (Konzentration XXX) are added. After flow through (about 2 minutes) and 4 minutes additional incubation time 2 times 750µl washing buffer are added to wash out unbound material.

To adjust the PH for the substrate reaction 500µl substrate buffer are added. After complete run-through TMB substrate is added.

The dye formation is stopped after 6 minutes by adding 750µl substrate buffer again.

### Read out

The read out can be done in two ways: a direct readout using the Senova Photometer can be done right after stopping the precipitation process. If Senova Abicap Photometer is not available standard ELISA Reader can be used after elution. Abicap Frames are adopted to 96 ELISA Format to make Elution as easy as possible.

### Senova Photometer

direct read out: The ABICAP column reader was designed for the direct read out. Blank the reader using a non stained reference column [general reference] and measure all columns optical density with that reference. The second way is to calibrate against each column before adding substrate which is the more precise method.

### Elisa Reader (Elution)

read out after elution: It is possible to elute the precipitated TMB into an microtiter plate by adding 150 µl 8% sulfuric acid and to determine optical density microtiter photometer (450 nm / 620 nm)

fig 1: completed assay

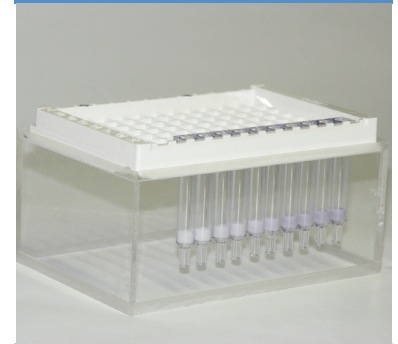
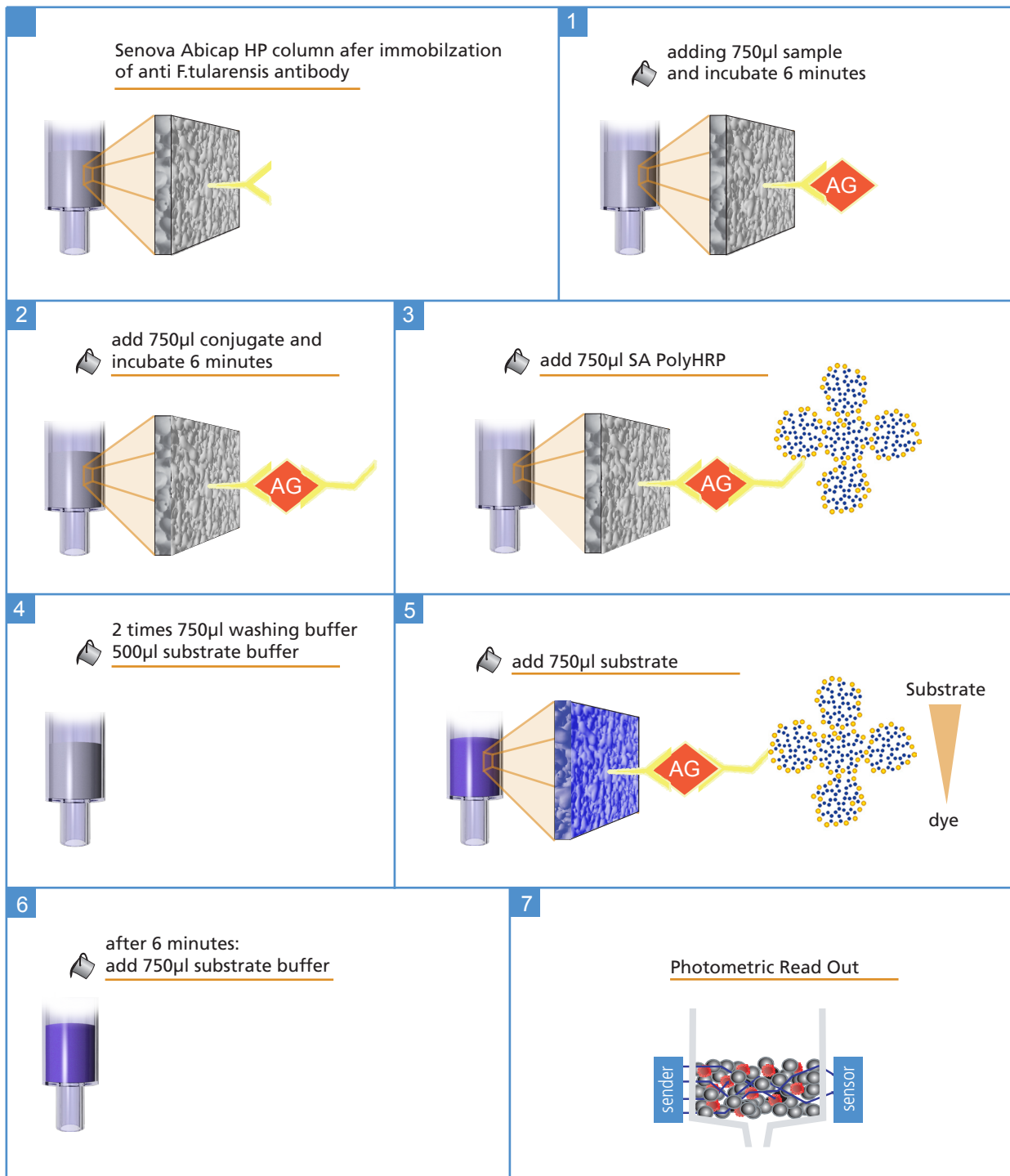
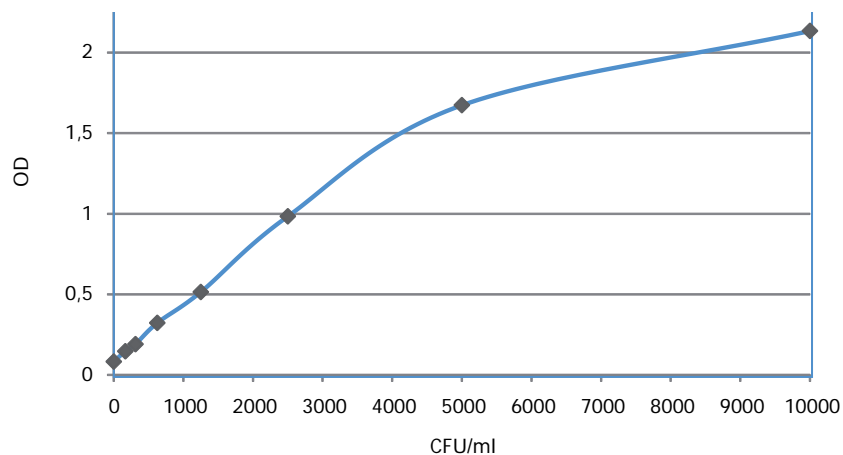


fig 1: Senova Photometer



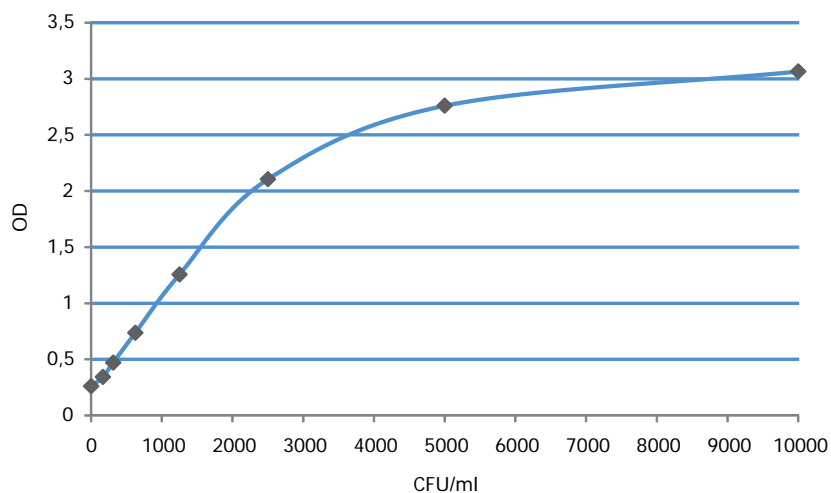


### direct read out (Senova Photometer)



CFU/ml	0	166	312	625	1250	2500	5000	10000
OD1	0,088	0,157	0,195	0,296	0,501	0,977	1,609	2,072
OD2	0,078	0,137	0,187	0,350	0,528	0,991	1,738	2,196
MW	0,083	0,147	0,191	0,323	0,515	0,984	1,674	2,134

### indirect read out (elution)



CFU/ml	0	166	312	625	1250	2500	5000	10000
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## conclusion

In this application note the approach for the production and use of senova HP Abicap columns for enzymatic immuno assay application as demonstrated.

The detection limit for the model analyte Francisella Tularensis in the order of 100 CFU/ml is comparable to a standard Elisa using the same set of reagents.

The dynamic range is about 1½ orders of magnitude which is typical for a single Abicap Filter arrangement.<sup>1</sup> The total assay time is about 40 Minutes. In dependence on the individual kinetics of analytes and antibodies assay time may be different. This is also true for reagents concentration and volumes.

Details of the anti F.tularensis ABICAP test performance were published in: Literatur (Grunow...)

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1 widening of dynamic range is possible using a multifilter arrangement (patent No: XXX)

