Pregnancy-associated plasma protein-A (PAPP-A)

Pregnancy-associated plasma protein-A (PAPP-A) is a metalloprotease that belongs to the metzincin superfamily of zinc peptidases. Its main substrate is the insulin-like growth factor binding protein-4 (IGFBP-4). This cleavage causes the release of bound IGF, which plays an important role in promoting cell differentiation and proliferation. PAPP-A was first identified in the serum of pregnant women, hence its name. It was later shown to be expressed in multiple tissues.

Two forms of PAPP-A

Heterotetrameric PAPP-A (htPAPP-A) is a screening marker for Down syndrome. htPAPP-A levels in maternal serum increases with gestational age until the full-term. If the concentration of htPAPP-A in the first trimester is markedly decreased, this indicates a higher risk of Down syndrome (1). htPAPP-A is a protein complex that consists of two PAPP-A subunits and two proforms of eosinophil basic proteins (proMBP) that are covalently linked to each other. proMBP has been shown to inhibit the protease activity of PAPP-A in this heterotetrameric complex (2).

Homodimeric PAPP-A (dPAPP-A) is abundantly expressed in unstable coronary atherosclerotic plaques (3). dPAPP-A circulates as a homodimer and not in a complex with proMBP. Based on several studies, dPAPP-A has been considered to be a promising marker of plaque destabilization in patients with acute coronary syndrome (ACS). Unfortunately, dPAPP-A assays have been shown to also detect htPAPP-A, which is the Down syndrome marker that is not related to atherosclerotic plaques. To prevent this, a dPAPP-A assay should be designed to only recognize dPAPP-A and not cross-react with htPAPP-A.

Another limitation to the use of dPAPP-A as a cardiac marker is the fact that the measurements were shown to be affected by heparin, which is an anti-coagulation agent often used as part of the treatment procedure with patients suffering from acute myocardial infarction. Therefore, to use dPAPP-A as a cardiac biomarker, the heparin injections should be taken into account when analyzing the samples. A promising surrogate marker for dPAPP-A is its main substrate, IGFBP-4. For more information, please see our IGFBP-4 TechNotes.

Reagents for immunoassay development

We provide monoclonal antibodies (MAbs) that are specific to PAPP-A and proMBP that allow for the development of highly sensitive, quantitative htPAPP-A immunoassays. We also provide reagents for the development of the dPAPP-A specific assay. In addition, we provide the dimeric recombinant human PAPP-A antigen to be used in PAPP-A assays.

CLINICAL UTILITY

First trimester screening marker for Down syndrome

Marker of atherosclerotic plaque destabilization

MONOCLONAL ANTIBODIES SPECIFIC TO HTPAPP-A

We provide several different MAbs that are specific to htPAPP-A. Some of the MAbs recognize the PAPP-A subunit while some are specific to the proMBP part of the heterotetrameric complex.

Total PAPP-A and htPAPP-A sandwich immunoassays

All MAbs were tested in pairs in sandwich fluoroimmunoassays as capture and detection antibodies with both forms of the antigen – htPAPP-A and dPAPP-A. The antibody pairs that perform best in our in-house assays are listed in Table 1. Calibration curves for two suggested pairs are shown in Figure 1.

MONOCLONAL ANTIBODIES SPECIFIC TO DPAPP-A

We offer a few MAbs that only recognize dPAPP-A and do not cross-react with htPAPP-A.

Selective dPAPP-A sandwich immunoassay

The antibody pair PAPP52-PAPP30 specifically recognizes dPAPP-A. In this prototype assay, one MAb is specific to dPAPP-A (Cat.# 4PD4), while the other MAb recognizes all known forms of PAPP-A (Cat.# 4P41). This prototype assay was tested with dPAPP-A purified from atherosclerotic coronary arteries, as well as with purified native htPAPP-A and human recombinant wild-type dPAPP-A (in-house preparation). The assay was able to recognize dimeric forms of the antigen with high specificity and with negligible cross-reactivity (< 1 %) with htPAPP-A. This MAb combination could be used for the development of a highly sensitive sandwich immunoassay that is suitable for the selective quantitative measurements of dPAPP-A in human blood.

dPAPP-A levels in the blood of patients with ACS

We measured the concentration of dPAPP-A in the plasma obtained from 43 patients with ACS (acute myocardial infarction, unstable angina) using the prototype assay PAPP52-PAPP30. The samples were withdrawn 3-20 hours following the onset of chest pain. As a control, we used plasma samples obtained from 34 non-ACS patients. The dPAPP-A levels in plasma from ACS patients were 2.77-fold higher than the plasma of the control group (P<0.0005) (Figure 2).

Table 1.
Recommended pairs for htPAPP-A and total PAPP-A sandwich immunoassay.

Detection of human htPAPP-A antigen (capture – detection)	Detection of total PAPP-A (htPAPP-A and/or dPAPP-A) (capture – detection)
10E2cc - 5H9	10E2cc - 10E1cc
5H9 - 10E2cc	4G11 - 3C8
5H9 - 7A6	4G11 - 10H9
10E1cc - 11E4	10E1cc - 7A6

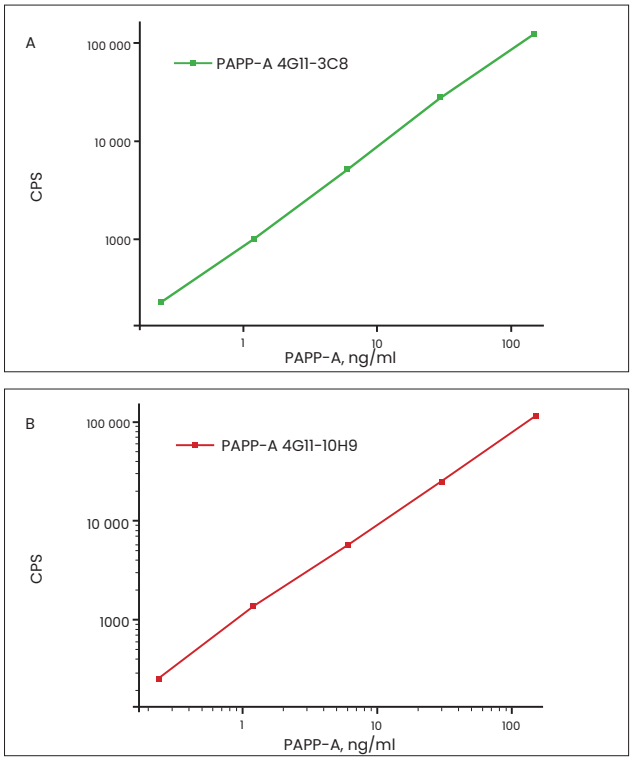

Figure 1.
Calibration curves for two PAPP-A sandwich immunoassays. (A) 4G11 – 3C8 and (B) 4G11 – 10H9.
Capture MAb: 4G11 (biotinylated)
Detection MAbs: 3C8 or 10H9 (labeled with stable Eu^{3+} -chelate)
Antigen: native htPAPP-A
Mixture of antibodies and antigen was incubated for 30 minutes at room temperature in streptavidin-coated plates.

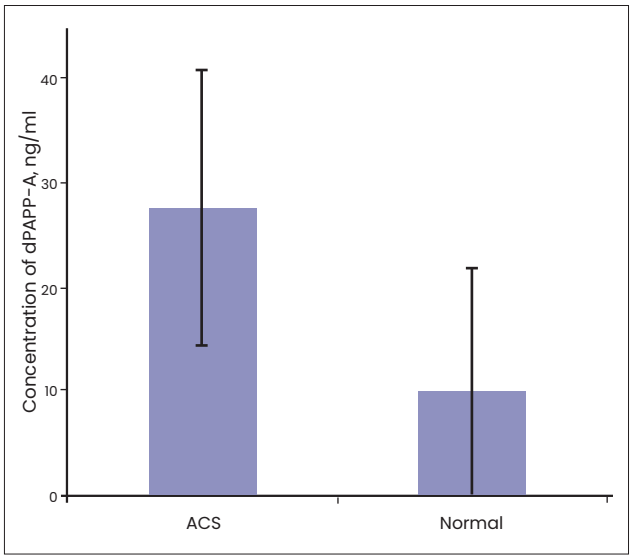

Figure 2.
dPAPP-A concentration in plasma samples of 43 ACS patients (ACS) and 34 non-ACS patients control group (Normal) measured by PAPP52 - PAPP30 sandwich immunoassay (mean+/-SD).
Capture MAb: PAPP52
Detection MAb: PAPP30 (labeled with Eu^{3+} chelate)
Incubation volume: 100 µl.
Incubation time: 30 min at room temperature.

Dimeric recombinant human PAPP-A

Our dimeric recombinant human PAPP-A has E483A mutation for stabilization of the protein due to the suppression of proteolytic activity and autocleavage. The dimeric recombinant PAPP-A antigen contains His10-tag and is expressed in mammalian cells. The product is purified by metal affinity chromatography. The protein presentation with 5% sucrose is optimized for storage in lyophilized form. The purity of the protein is >90% (Figure 3).

Recombinant human dimeric PAPP-A is immuno-chemically active in different sandwich immunoassay pairs, (Figure 4).

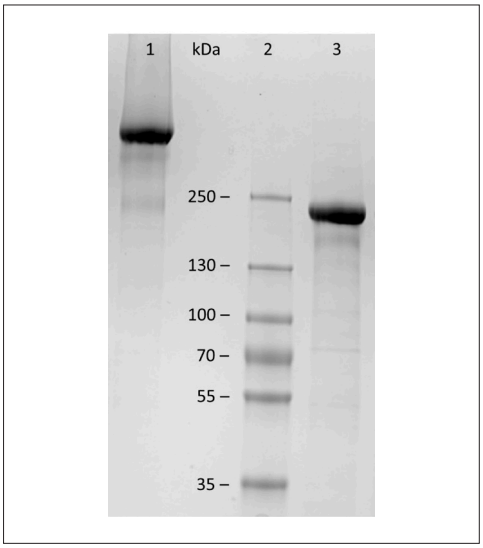

Figure 3. ***SDS-PAGE of recombinant human dimeric PAPP-A.***

1. in non-reducing conditions, 5 µg;
2. molecular weight markers;
3. in reducing conditions, 5 µg.

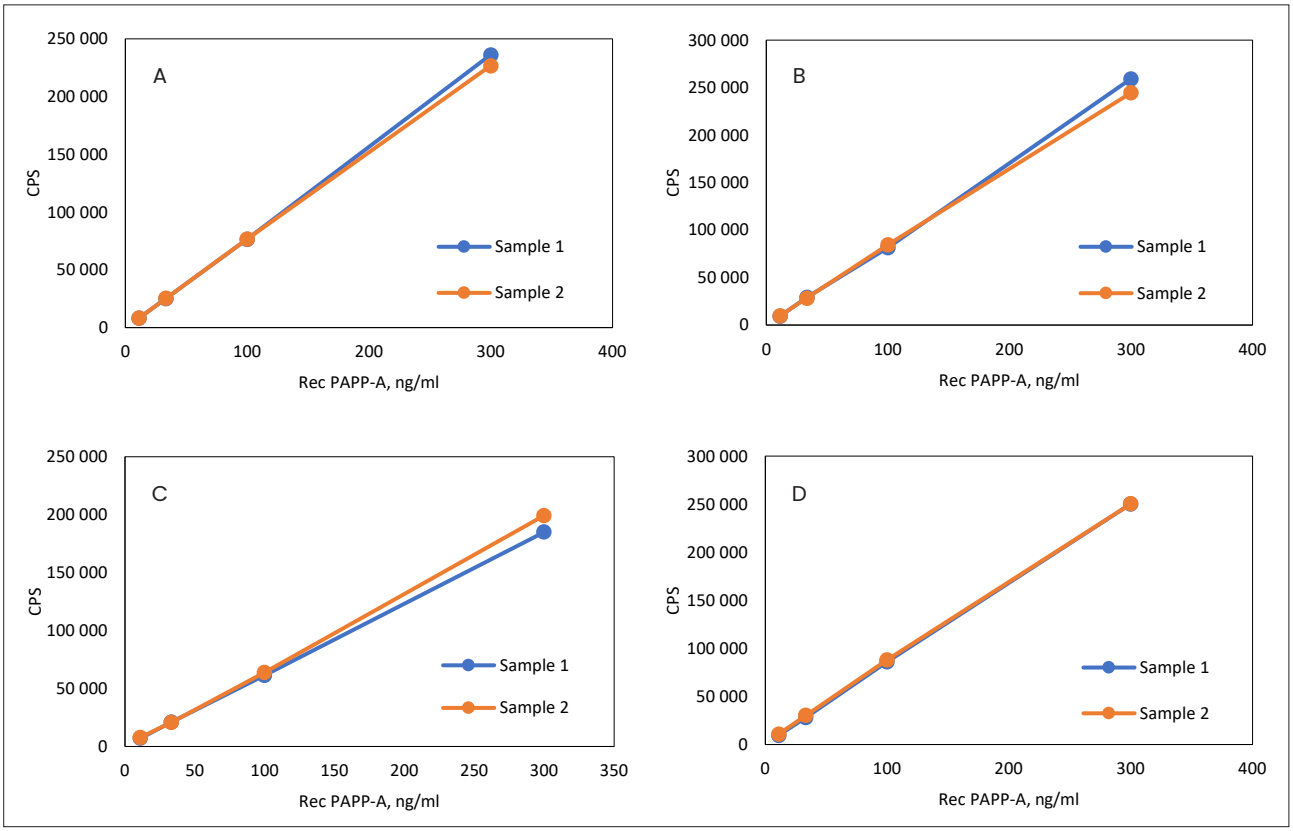

Figure 4.

Calibration curve for the recombinant human dimeric PAPP-A. *With A) 10A5-3C8, B) 10E2cc-10E1cc, C) 4G11-10H9, and D) 10E1cc-7A6 (capture-detection) sandwich immunoassays. The capture antibodies were adsorbed on the immunoassay microplates. A mixture of antigen and detection antibodies labelled with Eu^{3+} stable chelate was incubated for 30 minutes at room temperature. Samples 1 and 2 were independently produced recombinant human dimeric PAPP-A antigen samples.*

PAPP-A immunodetection in Western blotting

The MAbs 3C8 and 7A6 recognize the PAPP-A subunit whereas the MAbs 5H9 and 11E4 recognize the proMBP subunit of htPAPP-A in Western blotting after SDS-PAGE in reducing and non-reducing conditions (Figure 5). The MAbs 4G11 and 10E1cc recognize htPAPP-A in Western blotting only after electrophoresis in non-reducing conditions (data for 4G11 not shown here).

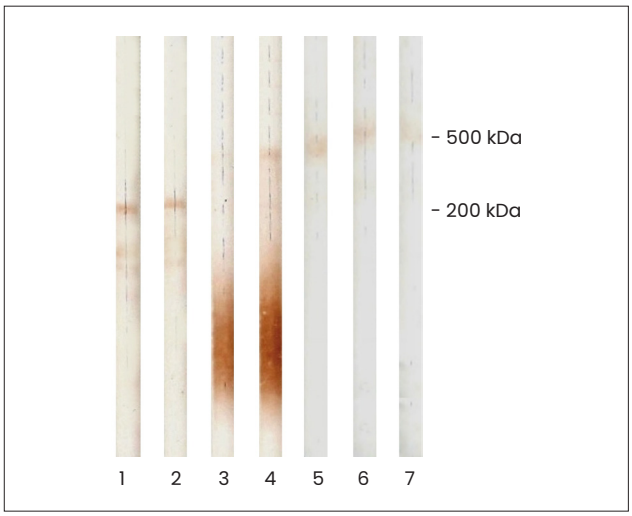

Figure 5. Detection of human PAPP-A and proMBP subunits of htPAPP-A by monoclonal antibodies in Western blotting.
Lane 1: 7A6
Lane 2: 3C8
Lane 3: 5H9 (proMBP-specific)
Lane 4: 11E4 (proMBP-specific)
Lane 5: 7A6
Lane 6: 3C8
Lane 7: 10E1
Lanes 1-4: after SDS-PAGE in reduction conditions.
Lanes 5-7: after SDS-PAGE in non-reducing conditions. The heterotetrameric complex was detected by anti-PAPP-A MAbs.

REFERENCES

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2. **Overgaard, MT., Haaning, J., Boldt, HB., Olsen, IM., Laursen, LS., et al.** Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor.// J Biol Chem; 275:31128-33 (2000).
3. **Bayes-Genis, A., Conover, C. A., Overgaard, M. T., Bailey, K. R., Christiansen, M., Holmes, D. R. Jr, et al.** Pregnancy-associated plasma protein A as a marker of acute coronary syndromes.// N Engl J Med, 345 (14), 1022-9 (2001).

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Pregnancy-associated plasma protein A (PAPP-A), human	4P41cc	10E1cc	IgG2b	*In vitro*, EIA, WB, PAPP-A subunit
		10E2cc	IgG2b	*In vitro*, EIA, PAPP-A subunit
	4P41	5H9	IgG2b	EIA, proMBP subunit
		4G11	IgG2a	EIA, WB, PAPP-A subunit
		3C8	IgG2a	EIA, WB, PAPP-A subunit
		10H9	IgG2a	EIA, PAPP-A subunit
		11E4	IgG2b	WB, proMBP subunit
		7A6	IgG2a	EIA, PAPP-A subunit
		PAPP52	IgG1	EIA, PAPP-A subunit
Dimeric form of pregnancy-associated plasma protein A (dPAPP-A), human	4PD4	PAPP30	IgG1	EIA, dimeric form of PAPP-A only

ANTIGEN

Product name	Cat. #	Purity	Source
PAPP-A, human, recombinant	8PA1	>90%	Recombinant

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

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