TechNotes

HyTest

Clinical and Research Area | Fertility and Pregnancy | Cardiac Markers

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 insulin-like growth factor binding protein (IGFPB) 4. This cleavage causes release of bound IGF, which plays an important role in promoting cell differentiation and proliferation. PAPP-A was first identified from the serum of pregnant women, hence its name. Later, it was 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 level in maternal serum increases with gestational age until 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 consisting of two PAPP-A subunits and two proforms of eosinophil basic proteins (proMBP) covalently linked to each other. proMBP has been shown to inhibit the protease activity of PAPP-A in this heteromeric complex (2).

CLINICAL UTILITY

- ✓ First trimester screening marker for Down syndrome
- ✓ Marker of atherosclerotic plaque destabilization

Homodimeric PAPP-A (dPAPP-A) is abundantly expressed in unstable coronary atherosclerotic plaques (3). dPAPP-A circulates as a homodimer and not in 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, the Down syndrome marker not related to atherosclerotic plaques. In order to prevent this, a dPAPP-A assay should be designed so that it only recognizes dPAPP-A and does 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, an anti-coagulation agent often used as part of the treatment procedure with patients suffering from acute myocardial infarction. So in order 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) specific to PAPP-A and proMBP that allow for the development of highly sensitive, quantitative htPAPP-A immunoassays. We also provide a selection of MAbs that only detect dPAPP-A and do not cross-react with htPAPP-A.

In addition, we provide htPAPP-A antigen purified from retroplacental blood. HyTest is the largest global supplier of this product.

Monoclonal antibodies specific to htPAPP-A

We provide several different MAbs 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 performing best in our in-house assays are listed in Table 1. Calibration curves for two suggested pairs are shown in Figure 1.

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)
10E2 - 5H9	10E2 - 10E1
5H9 - 10E2	4G11 - 3C8
5H9 - 7A6	4G11 - 10H9
10E1 - 11E4	10E1 - 7A6

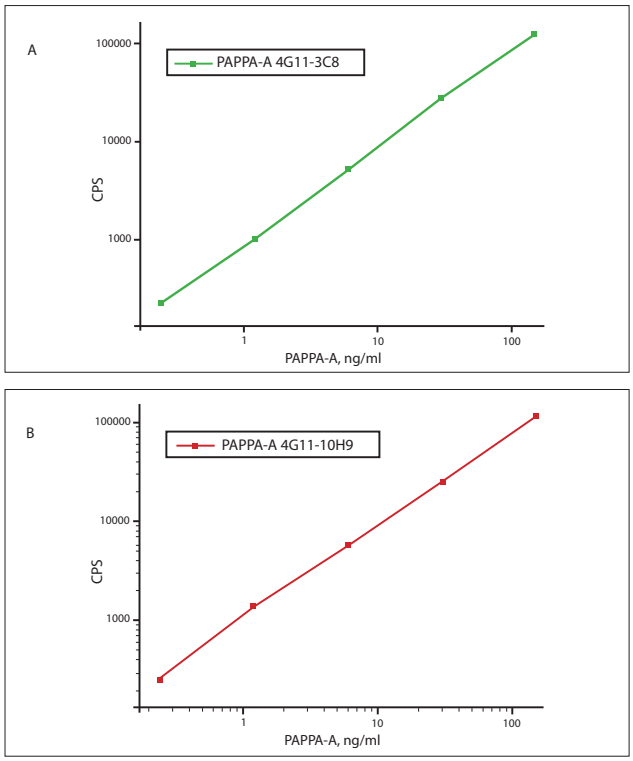

Figure 1. Calibration curves for two PAPP-A sandwich immunoassays. (A) 4G11 – 3C8 and (B) 4G11 – 10H9.
Capture MAb: 4G11 (biotinylated)
Detection MAbs: 3C8 or 10H9 (labeled with stable Eu^{3+} -chelate)
Antigen: htPAPP-A
Mixture of antibodies and antigen was incubated for 30 minutes at room temperature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

PAPP-A immunodetection in Western blotting

MAbs 3C8 and 7A6 recognize PAPP-A subunit whereas MAbs 5H9 and 11E4 recognize the proMBP subunit of htPAPP-A in Western blotting after SDS-PAGE in reducing and non-reducing conditions. MAbs 4G11 and 10E1 recognize htPAPP-A in Western blotting only after electrophoresis in nonreducing conditions (see Figure 2 and data not shown here).

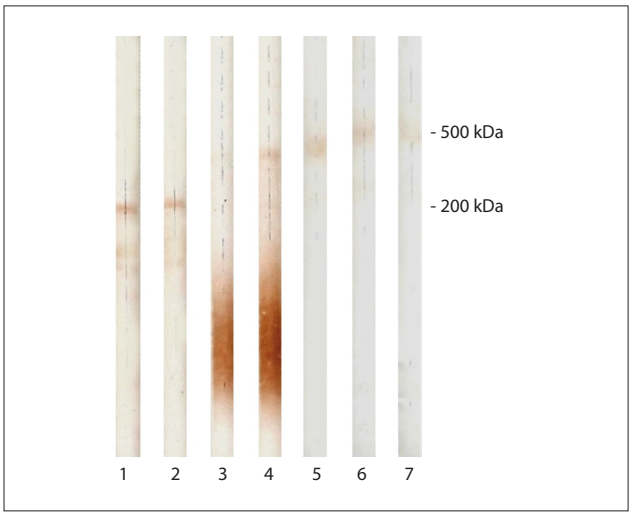

Figure 2. Detection of human PAPP-A and proMBP subunits of htPAPP-A by monoclonal antibodies in Western blotting.
Lane 1: 7A6
Lane 2: 3C8
Lane 3: 5H9 (proMBP-specific)
Lane 4: 11E4 (proMBP-specific)
Lane 5: 7A6
Lane 6: 3C8
Lane 7: 10E1
Lanes 1-4: after SDS-PAGE in reduction conditions.
Lanes 5-7: Non-reducing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

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

We recommend two MAb combinations for the development of dPAPP-A sandwich immunoassay (Table 2). In these prototype assays one of the MAbs is specific to dPAPP-A (Cat.# 4PD4), while the other MAb can recognize all known forms of PAPP-A (Cat.# 4P41). The recommended combinations were tested with dPAPP-A purified from atherosclerotic coronary arteries, as well as with purified htPAPP-A (Cat.# 8P64) and human recombinant dPAPP-A (in-house preparation). The prototype assays were able to recognize dimeric forms of the antigen with high specificity and with negligible cross-reactivity (< 1 %) with htPAPP-A. These MAb combinations could be used for the development of highly sensitive sandwich immunoassays that are suitable for the selective quantitative measurements of dPAPP-A in human blood.

Table 2. Recommended pairs for dPAPP-A sandwich immunoassay.

Capture	Detection
PAPP52	PAPP30
PAPP2	7A6

Figure 3 shows the calibration curves for the PAPP2-7A6 sandwich fluoroimmunoassay. The detection limit of the immunoassay was better than 0.3 ng/ml with human recombinant dPAPP-A (in-house preparation) used as a calibrator. This assay revealed very low (< 1 %) cross-reactivity with the heterotetrameric form of PAPP-A.

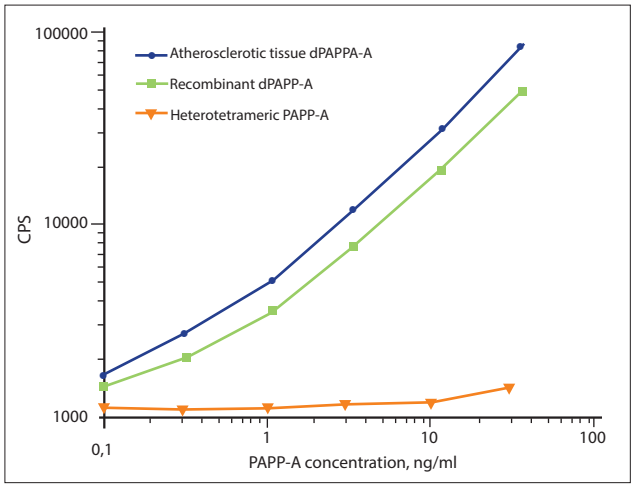

Figure 3. Calibration curves for a dPAPP-A immunoassay.
Capture MAb: PAPP2
Detection MAb: 7A6 (labeled by Eu^{3+} chelate)
Incubation volume 100 µl.
Incubation time: 30 min at room temperature.

dPAPP-A levels in the blood of patients with ACS

We measured the concentration of dPAPP-A in the plasma 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 in plasma from the control group (P<0.0005) (see Figure 4).

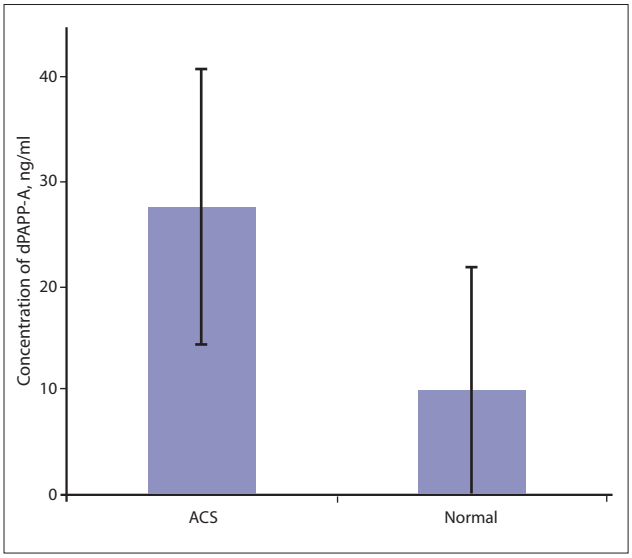

Figure 4. dPAPP-A concentration in plasma samples of 43 ACS patients (ACS) and 34 non-ACS patients control group (Normal) measured by PAPP52 - PAPP30 sandwich immunoassay (mean+/-SD).
Capture MAb: PAPP52
Detection MAb: PAPP30 (labeled with Eu^{3+} chelate)
Incubation volume: 100 µl.
Incubation time: 30 min at room temperature.

Heterotetrameric PAPP-A/proMBP complex (htPAPP-A)

HyTest's htPAPP-A is purified from the pooled retroplacental blood and purity is over 85% according to SDS-PAGE (Figure 5). htPAPP-A is recognized by monoclonal antibodies specific to different parts of PAPP-A or proMBP (Cat # 4P41). Antigen can be used as a calibrator for total PAPP-A and htPAPP-A sandwich immunoassays.

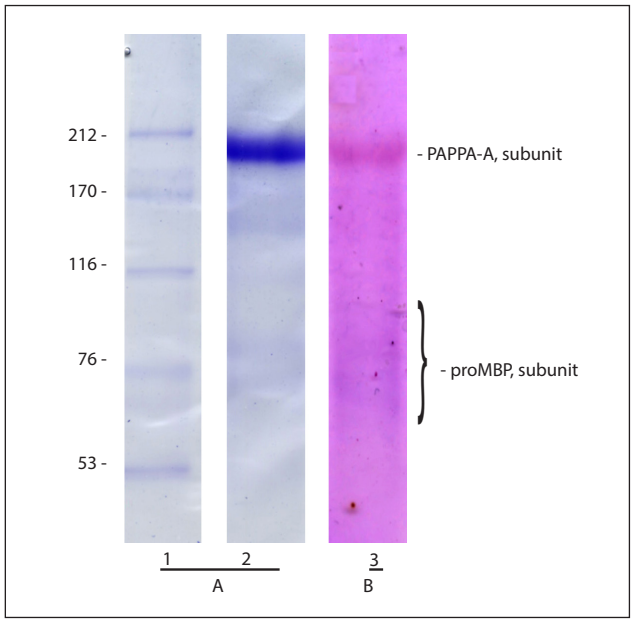

Figure 5. SDS-gel electrophoresis of htPAPP-A in reducing conditions.
Lane 1: molecular weight standards
Lanes 2, 3: human htPAPP-A
Antigen loaded: 5 µg
Gel staining: **A**: Coomassie brilliant blue R-250, **B**: Stains all (staining of glycosylated proteins).
Comments: proMBP subunit migrates in gel as a diffuse band with molecular mass about 50-90 kDa and is not stained by Coomassie brilliant blue because of high degree of glycosylation (~40%).

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Pregnancy-associated plasma protein A (PAPP-A), human	4P41	10E1	IgG2b	EIA, WB, PAPP-A subunit
		10E2	IgG2b	EIA, PAPP-A subunit
		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
Pregnancy-associated plasma protein A (PAPP-A), human, *in vitro*	4P41cc	10E1cc	IgG2b	EIA, WB, PAPP-A subunit
		10E2cc	IgG2b	EIA, PAPP-A subunit
Dimeric form of pregnancy-associated plasma protein A (dPAPP-A), human	4PD4	PAPP2	IgG1	EIA, dimeric form of PAPP-A only
		PAPP30	IgG1	EIA, dimeric form of PAPP-A only

ANTIGEN

Product name	Cat. #	Purity	Source
PAPP-A, heterotetrameric form (htPAPP-A)	8P64	>85%	Pooled retroplacental blood

References

1. **Palomaki GE, Lambert-Messerlian GM, Canick JA** A summary analysis of Down syndrome markers in the late first trimester.// Adv Clin Chem. 2007;43:177-210.
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).

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