TechNotes

HyTest

Clinical and Research Area | Inflammation

Procalcitonin (PCT)

Procalcitonin (PCT) is a small protein (~13 kDa) that is synthesized by the C-cells of the thyroid glands. It is considered to be the main marker of disorders that are accompanied by systemic inflammation and sepsis.

PCT is encoded by the CALC-1 gene and is the precursor of the calcitonin hormone. It is produced from a 141 amino acid long pre-procalcitonin. After the removal of the signal peptide (amino acids 1-25), the 116 amino acid long PCT undergoes successive cleavages to form three molecules: the N-terminal fragment (N-terminal PCT, 57 amino acid residues (a.a.r.)), calcitonin (32 a.a.r.), and katacalcin (21 a.a.r.) (Figure 1).

PCT belongs to a family of related proteins (the CAPA peptides family), which also includes calcitonin, the calcitonin gene-related peptides I and II, an amylin and an adrenomedullin.

CLINICAL UTILITY

- ✓ Systemic inflammation
- ✓ Sepsis

PCT in diagnostics

In 1993, an elevated level of PCT in patients with a system infection of bacterial origin was reported for the first time (1). It was shown that "inflammatory" PCT is not produced in C-cells, but rather in all parenchymal tissues and the differentiated cell types (2-4). PCT is a good marker of bacterial infection because its level in the blood of normal subjects is very low and due to the fact that viral infections cause only a minor increase in PCT concentration. In addition, the diagnostic value of PCT is further supported by the close correlation between PCT concentration and the severity of inflammation (1, 5).

In some cases, an increase in PCT concentration may be induced by factors independent of sepsis and infection. Surgery, polytrauma, heat shock, burn injuries, and cardiogenic shock also lead to an increase in the PCT level (1). Furthermore, the

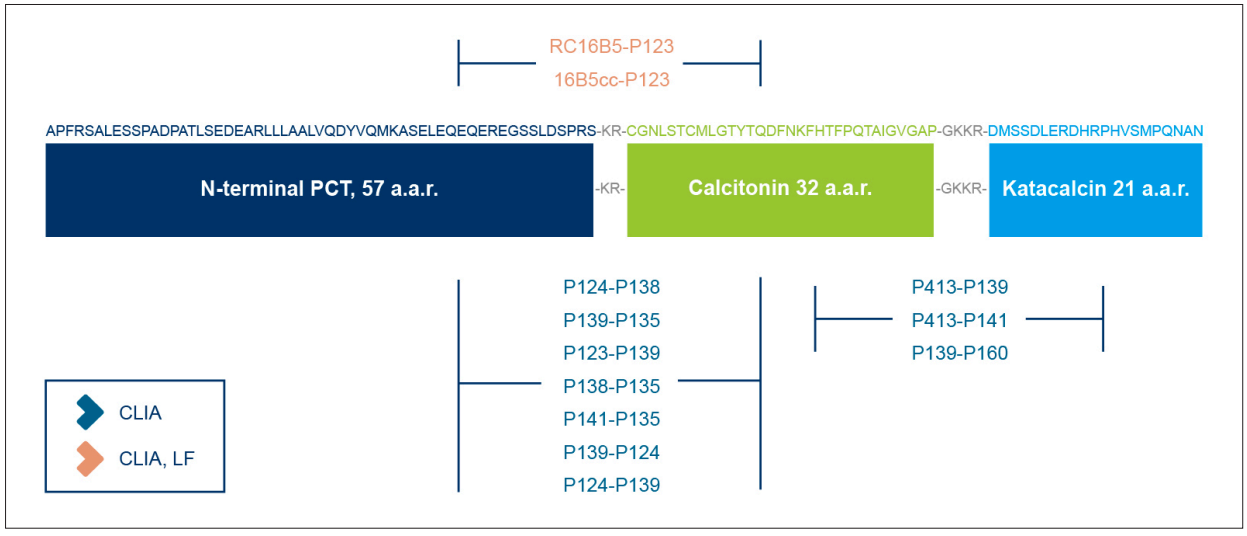

Figure 1. The amino acid sequence of human procalcitonin (116 a.a.r.), representing N-terminal PCT (1-57), calcitonin (60-91), and katacalcin (96-116), and pairs of mAbs recommended for PCT sandwich immunoassays (capture-detection).

importance of monitoring the PCT level changes following cardiac surgery or heart transplantation for differentiating acute graft rejection from bacterial or fungal infections has been confirmed in multiple studies (5).

Assay development and pair recommendations

For the development of PCT immunoassays, HyTest offers monoclonal antibodies (mAbs) that are specific to different fragments of the PCT molecule: N-terminal fragment of PCT, calcitonin, and katacalcin. These mAbs can be used for the detection of the full-length or partially processed PCT molecule by using pairs of antibodies that are specific to different parts of PCT. The specificity of antibodies and the recommended capture-detection pairs for sandwich immunoassays are shown in Table 1. In addition to the mAbs, we also have a polyclonal PCT antibody that can be used for PCT-specific immunoassay development. HyTest also offers a recombinant, full-length PCT antigen that can be used as a calibrator in PCT or calcitonin immunoassays.

The best sensitivity was achieved using different NT-PCT – Calcitonin combinations of mAbs: P124-P138, P139-P135, and P123-P138 (Table 1). However, most of the mAbs could be used in different combinations and suitable combinations should be evaluated separately for different platforms, including CLIA and LF. All systems show either no or very low cross-reaction with isolated calcitonin, katacalcin, and calcitonin gene-related peptide-1 and peptide-2 (CGRP1 and CGRP2). The data is shown in Table 2.

Table 1. Anti-PCT sandwich immunoassay pair recommendations in CLIA and LF. Limit of detection (LoD). N, C, and K represent NT-PCT, calcitonin, and katacalcin respectively. Correlation data with the Roche Elecsys® BRAHMS PCT assay were obtained by using three separate collections of patient samples (n=34, 46, and 103).

Preferred pair recommendations				
Capture-detection	**Specificity (capture-detection)**	**LoD [pg/mL]**	**Correlation**	**Application**
P124-P138	N-C	0.27	0.9961	CLIA
P139-P135	C-N	0.28	0.9880	CLIA
P123-P138	N-C	0.28	0.9998	CLIA
P138-P135	C-N	0.53	0.9964	CLIA
P141-P135	C-N	0.68	0.9973	CLIA
P139-P124	C-N	0.73	0.9953	CLIA
P124-P139	N-C	0.87	0.9975	CLIA
P413-P139	K-C	1.43	0.9930	CLIA
RC16B5-P123	C-N	1.47	0.9846	CLIA, LF
P413-P141	K-C	1.82	0.9970	CLIA
Other pair recommendations				
P135-P138	N-C	0.35	0.9994	CLIA
P135-P139	N-C	0.42	0.9984	CLIA
P141-P223	C-N	0.78	0.9850	CLIA
P123-P139	N-C	1.09	0.9991	CLIA
P141-P160	C-K	1.40	0.9993	CLIA
P139-P223	C-N	1.43	0.9800	CLIA
16B5cc-P123	C-N	2.08	0.9523	CLIA, LF
P139-P160	C-K	2.32	0.9999	CLIA

Table 2. Anti-PCT sandwich immunoassay cross-reactivity data of mAb pairs. The CGRP1 and CGRP2 are calcitonin gene-related proteins 1 and 2. Calcitonin, katacalcin, CGRP1, and CGRP2 are used in 10 ng/mL in CLIA. The data are shown as a percentage of full-length PCT immunoreactivity. N, C, and K represent NT-PCT, calcitonin, and katacalcin respectively.

Pairs	**Specificity**	**Cross-reactivity, % (for 10 ng/mL in CLIA)**			
		Calcitonin	**Katacalcin**	**CGRP1**	**CGRP2**
P124-P138	N-C	<0.005	<0.005	<0.005	<0.005
P139-P135	C-N	0.0000	0.0000	0.0000	0.0000
P123-P138	N-C	<0.005	<0.005	<0.005	<0.005
P138-P135	C-N	<0.005	<0.005	<0.005	<0.005
P141-P135	C-N	<0.005	<0.005	<0.005	<0.005
P139-P124	C-N	<0.005	<0.005	<0.005	<0.005
P124-P139	N-C	<0.005	<0.005	<0.005	<0.005
P413-P139	K-C	0.0335	0.0141	0.0008	0.0042
RC16B5-P123	C-N	0.0231	0.0056	0.0060	0.0000
P413-P141	K-C	0.0278	0.0043	0.0092	0.0355
P135-P138	N-C	0.0170	0.0100	0.0030	<0.0050
P135-P139	N-C	<0.005	0.0070	0.0410	0.039
P141-P223	C-N	0.0924	0.0102	0.0201	0.0000
P123-P139	N-C	0.0297	0.0113	0.0038	0.0065
P141-P160	C-K	0.0154	0.0000	0.0057	0.0069
P139-P223	C-N	0.0095	0.0067	0.0085	0.0088
16B5-P123	C-N	0.0175	0.0035	0.0023	0.0066
P139-P160	C-K	0.0206	0.0046	0.0041	0.0030

The typical calibration curves of six detection systems are presented in Figure 2.

We also tested several assays for their ability to detect native PCT in human serum. Serum samples from two septic patients and one healthy individual were analyzed using different combinations of anti-PCT mAbs. The correlations of the developed assays with the Roche Elecsys BRAHMS PCT were calculated (Figure 3 and Table 1). To calculate the correlations of measurement results between our in-house PCT assays and the BRAHMS assay (Roche Elecsys BRAHMS PCT), we tested the serum collection of patients with sepsis and healthy donors. All samples were tested for PCT concentration on the Cobas 411 or the 6000 analyzers (Roche) and compared with our in-house PCT assays using the CLIA method.

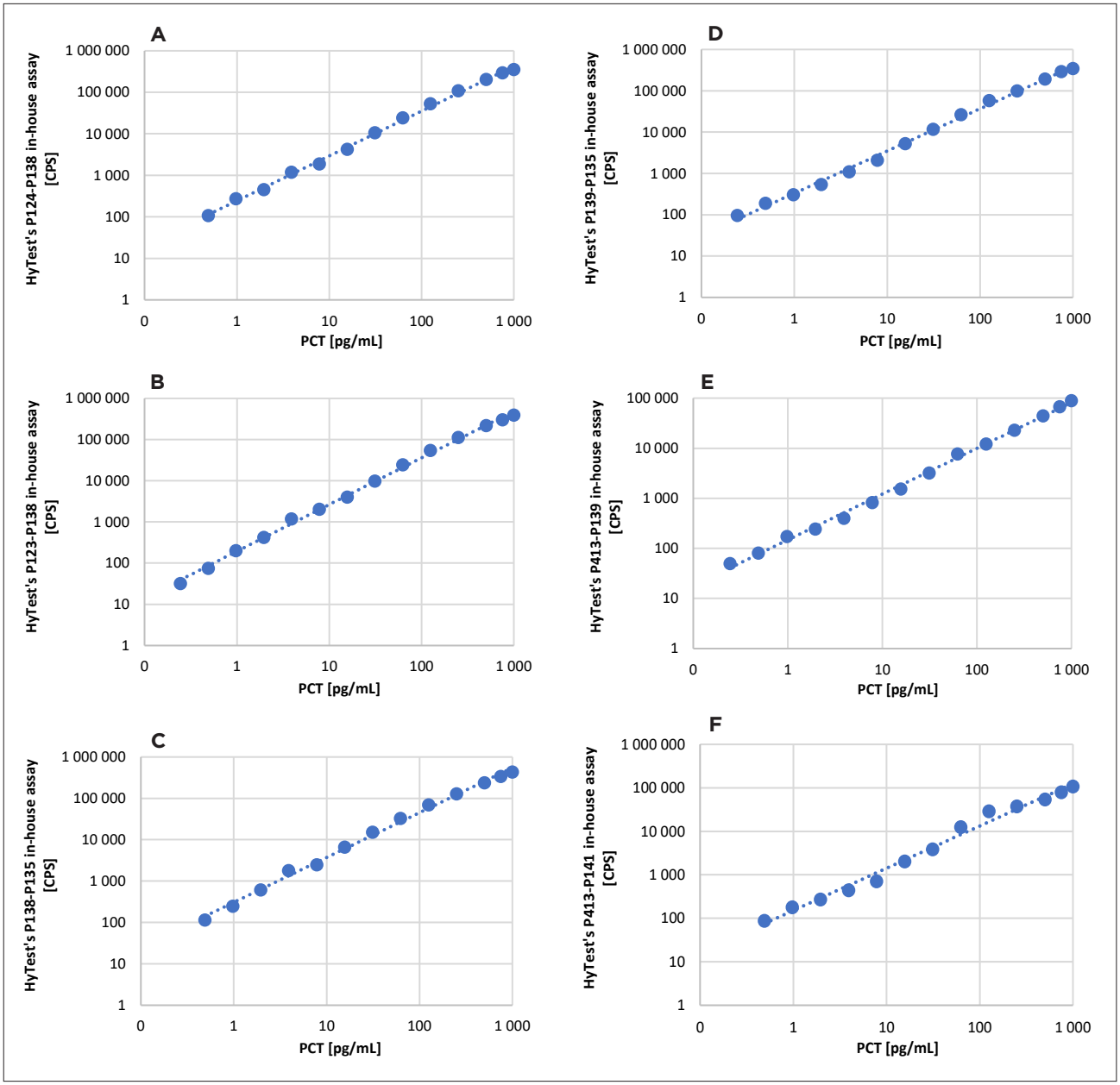

Figure 2. Calibration curves for six human PCT CLIA immunoassays utilizing antibodies with different epitope specificity.
A) P124-P138
B) P139-P135
C) P123-P138
D) P138-P135
E) P413-P139
F) P413-P141
Capture mAb: 0.05 µg/well
Detection mAb, biotinylated: 12.5 ng/well
Streptavidin-PolyHRP (ThermoFisher): 1:50000
Antigen: PCT human recombinant
Incubation time: 30 min

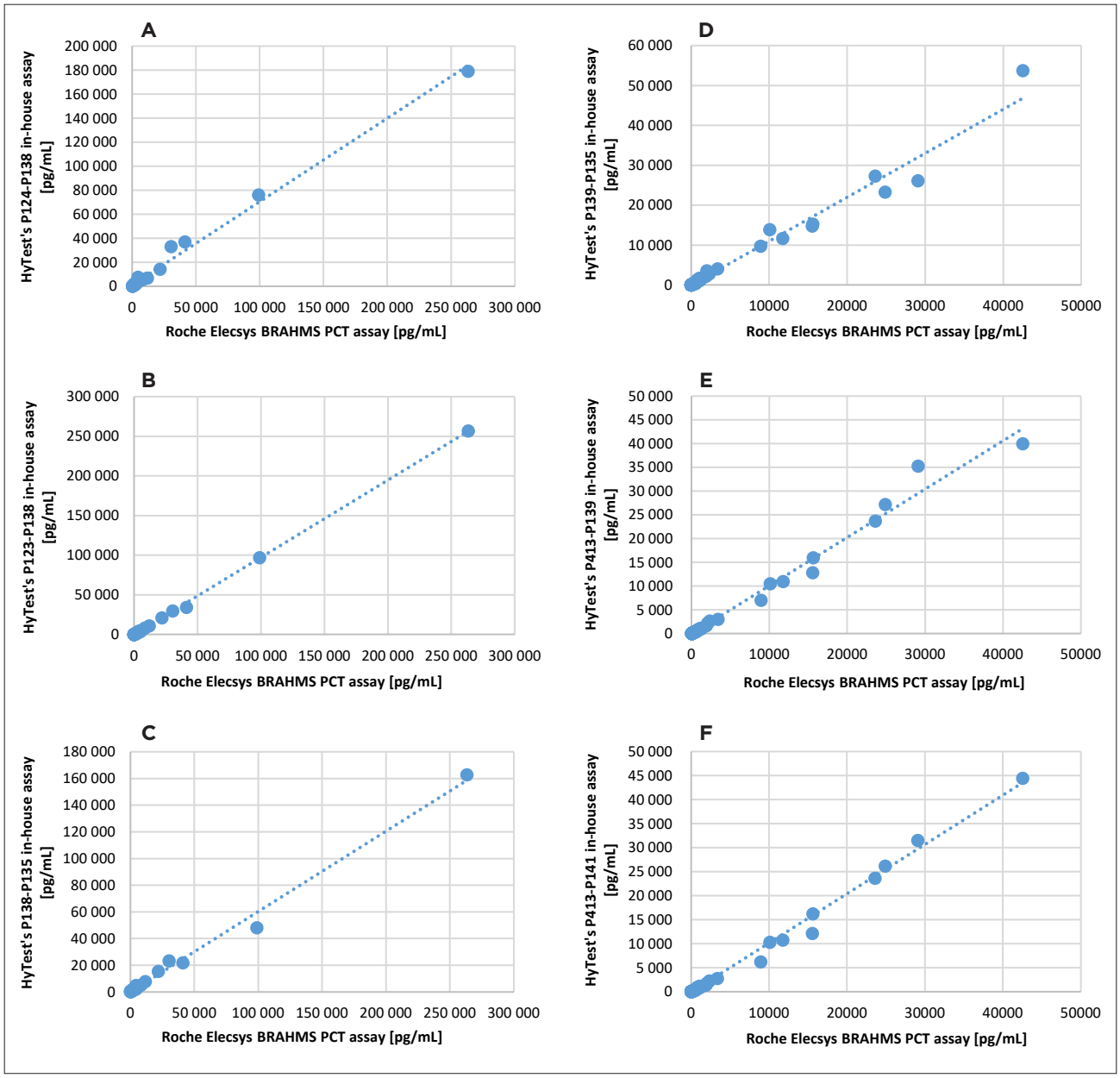

Figure 3. Correlation of the developed assays with the Roche Elecsys® BRAHMS PCT assay.
A) P124-P138 (n=34)
B) P123-P135 (n=46)
C) P138-P135 (n=34)
D) P139-P135 (n= 103)
E) P413-P139 (n= 103)
F) P413-P141 (n=103).
Correlations A-C) Capture mAb: 0.05 µg/well
Detection mAb, biotinylated: 12.5 ng/well
Streptavidin-PolyHRP (ThermoFisher): 1:50000
Incubation time: 30 min
Correlations D-F) Capture mAb coated on paramagnetic particles: 20 µg/well
Detection mAb, Acridinium ester-labeled: 30 ng/well
Incubation time: 10 min

Recombinant chimeric antibodies to PCT

HyTest has developed two anti-PCT mAbs in recombinant chimeric format with human IgG1 constant domains (RC16B5 and P223). Recombinant chimeric antibodies demonstrate similar sensitivity compared to original mouse antibodies and give the opportunity to avoid false-positive signals in human anti-mouse antibody (HAMA) and rheumatoid factor (RF) positive sera. The mouse-derived mAb 16B5cc and chimeric mAb RC16B5 were compared to ascertain the impact of these factors (Figure 4).

Polyclonal anti-procalcitonin antibody

HyTest offers polyclonal anti-human procalcitonin (Cat.# PPC3) obtained in goats. Human recombinant full-length procalcitonin was used as an immunogen. The immunoaffinity method, utilizing PCT-sepharose as an affinity matrix, was used for polyclonal antibody purification. The following sandwich pairs can be recommended with a polyclonal anti-procalcitonin antibody (Table 3).

Table 3. Capture-detection pair recommendations for polyclonal anti-human procalcitonin (Cat.# PPC3).

Capture	Detection
PPC3	16B5cc
PPC3	P139

Monoclonal antibodies specific to PCT

Some antibodies were also tested for their ability to detect PCT in Western blotting after SDS-electrophoresis in reducing conditions (Figure 5).

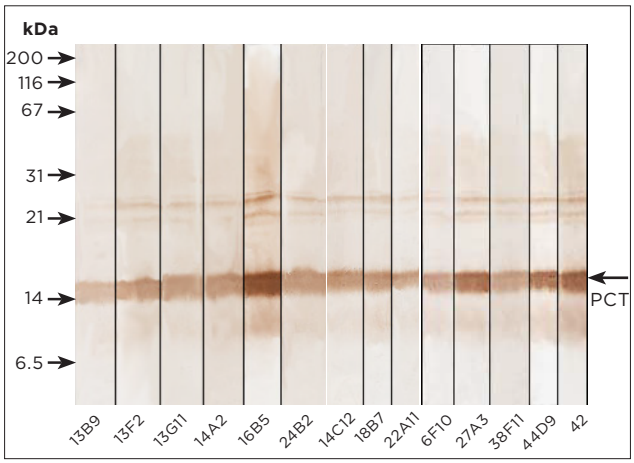

Figure 5. Detection of human recombinant PCT (100 ng/lane) by monoclonal antibodies that are specific to calcitonin, katacalcin, and the N-terminal fragment of PCT in Western blotting after 15% SDS-PAGE in reducing conditions.

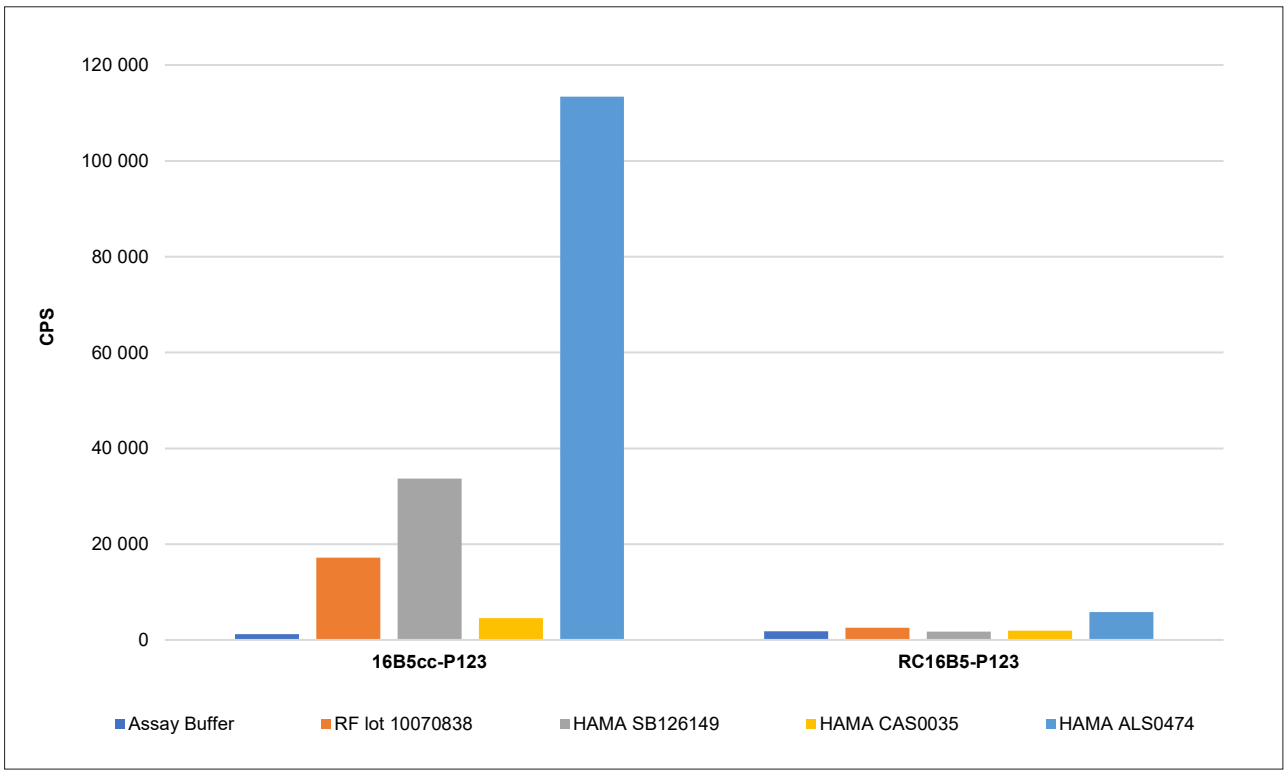

Figure 4. Comparison of the detecting systems 16B5cc-P123 and RC16B5-P123 tested with RF and HAMA positive sera (sera of the donors without sepsis).

Recombinant human procalcitonin

HyTest's recombinant human PCT (Cat.# 8PC5) is expressed in *E. coli* as a full-length, 116 amino acid polypeptide without a signal peptide and without tags (the sequence corresponds to UniProt P01258 lacking a signal peptide). It is purified by immunoaffinity and ion-exchange chromatographic methods with over 95% purity (see Figure 6). This recombinant PCT can be used as a calibrator in procalcitonin or calcitonin immunoassays.

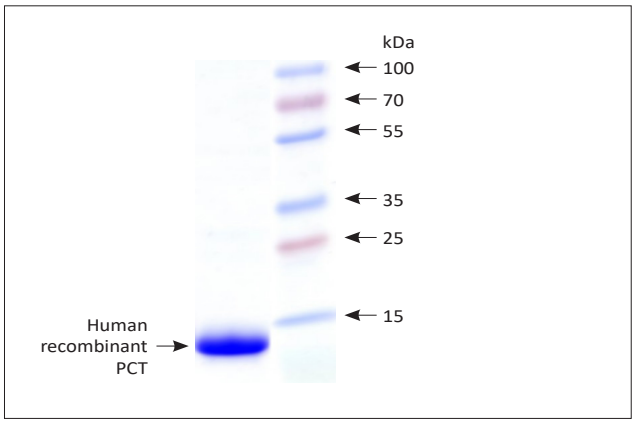

Figure 6. SDS-PAGE of purified human recombinant PCT (5 μg) in reducing conditions. Purity was determined by densitometry analysis of the gel.

Stability studies

To determine how well our recombinant antigen retains its immunoreactivity after dissolving the lyophilized product in the buffer, we made a 1 mg/ml solution in a 20 mM Tris, 150 mM NaCl, pH 8 buffer, and tested its performance after storing the dilution at different temperatures (Figure 7). It was also tested after repeated freeze-thaw cycles (Figure 8). Our results show that the antigen is robust and retained its activity well under the tested conditions.

Comparison study

We compared our recombinant, tag-free PCT (Cat.# 8PC5), with a recombinant, tag-free PCT from another supplier. Our results show that there was no difference in the immunoreactivity of these proteins (Figure 9).

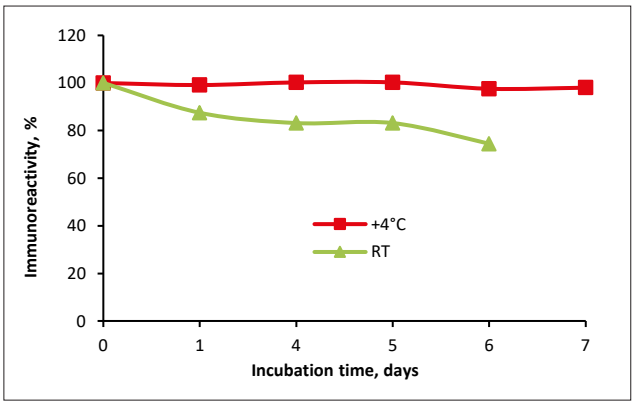

Figure 7. Stability of 1 mg/ml antigen solution at 4°C and room temperature (RT). PCT at 1 mg/ml concentration was incubated at +4°C or RT for the time periods indicated. Immunoreactivity was measured in sandwich ELISA with the mAb pair 16B5-42.

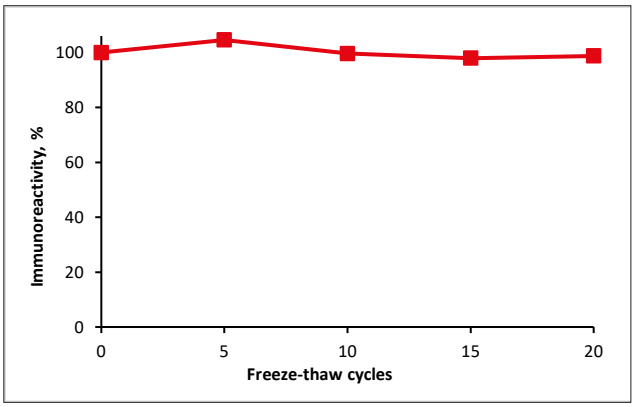

Figure 8. Stability of 1 mg/ml antigen solution after repeated freeze-thaw cycles. PCT at 1 mg/ml concentration was frozen at -70°C and thawed at RT for the indicated number of times. Immunoreactivity was measured in sandwich ELISA with the mAb pair 16B5-42.

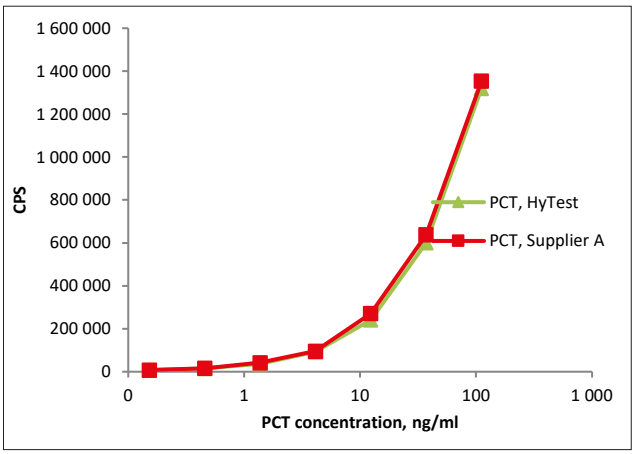

Figure 9. Comparative titration of tag-free PCT from HyTest and from Supplier A. Immunoreactivity was measured in sandwich ELISA using the MAb pair 16B5-42.

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Calcitonin	4C10cc	P138	IgG1	*In vitro*, CLIA, a.a.r. 72-81 of PCT
		P139	IgG1	*In vitro*, CLIA, a.a.r. 72-81 of PCT
		P141	IgG1	*In vitro*, CLIA, a.a.r. 72-81 of PCT
		RC16B5	IgG1	CLIA, LF, a.a.r. 72-81 of PCT, recombinant chimeric antibody
		13G11cc	IgG1	*In vitro*, EIA, WB, a.a.r. 72-81 of PCT
		14A2cc	IgG1	*In vitro*, EIA, WB, a.a.r. 72-81 of PCT
		16B5cc	IgG2b	*In vitro*, EIA, WB, a.a.r. 72-81 of PCT
		24B2cc	IgG1	*In vitro*, EIA, WB, a.a.r. 72-81 of PCT
	4C10	13B9	IgG2a	EIA, a.a.r. 60-69 of PCT
		13F2	IgG1	EIA, WB, a.a.r. 72-81 of PCT
Procalcitonin	4PC47	P123	IgG1	*In vitro*, CLIA, LF, a.a.r. 11-25 of PCT
		P223	IgG1	CLIA, a.a.r. 11-25 of PCT, recombinant chimeric antibody
		P124	IgG1	*In vitro*, CLIA, a.a.r. 11-25 of PCT
		P135	IgG2a	*In vitro*, CLIA, a.a.r. 11-25 of PCT
		P413	IgG2a	*In vitro*, CLIA, a.a.r. 96-105 of PCT, rat-mouse heterohybridoma antibody
		P160	IgG1	*In vitro*, CLIA, a.a.r. 102-108 of PCT
		44D9	IgG2a	EIA, WB
		6F10	IgG1	EIA, WB, a.a.r. 21-40 of PCT
		27A3cc	IgG2a	*In vitro*, EIA, WB, a.a.r. 21-40 of PCT
		38F11	IgG1	EIA, WB, a.a.r. 21-40 of PCT
		42cc	IgG2a	*In vitro*, EIA, WB, a.a.r. 21-40 of PCT
		22A11	IgG1	EIA, WB, a.a.r. 96-105 of PCT
		14C12cc	IgG1	*In vitro*, EIA, WB, a.a.r. 102-111 of PCT
		18B7	IgG1	EIA, WB, a.a.r. 102-111 of PCT

POLYCLONAL ANTIBODY

Product name	Cat. #	Host Animal	Remarks
Procalcitonin	PPC3	goat	EIA

ANTIGEN

Product name	Cat. #	Purity	Source
Procalcitonin, tag-free, recombinant	8PC5	>95%	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.

References

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