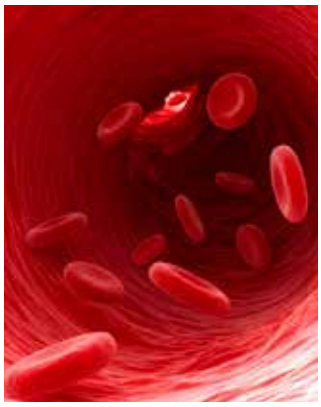
Procalcitonin (PCT)



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

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 because 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).

An increase in PCT concentration may in some cases 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). Further, the importance of monitoring 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).

CLINICAL UTILITY

- ✓ Systemic inflammation
- ✓ Sepsis

Assay development and pair recommendations

For the development of PCT immunoassays we offer monoclonal antibodies 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 Fig. 1. In addition to several antibodies, we also provide a recombinant, full length PCT antigen that can be used as a calibrator in PCT or calcitonin immunoassays.

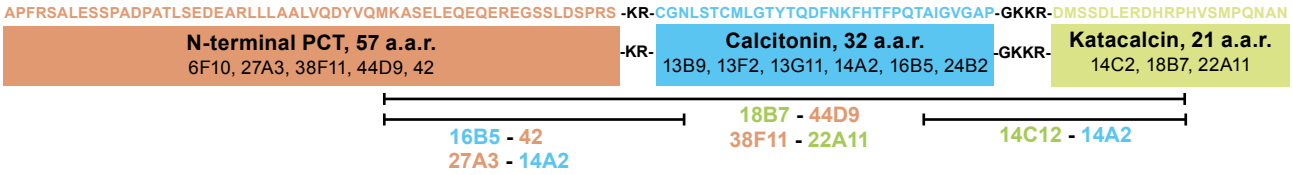


FIGURE 1. Amino acid sequence of human procalcitonin (116 a.a.r.), epitope specificities and pairs of mAbs recommended for PCT sandwich immunoassay (capture-detection).

Human procalcitonin, recombinant

Human recombinant PCT is expressed in *E. coli* as a full length, 116 amino acid polypeptide without a signal peptide and with no affinity tags (the sequence corresponds to UniProt P01258 lacking a signal peptide). It is purified by immunoaffinity and ion-exchange chromatographic methods. Over 95% purity is achieved (see Fig. 2). According to the MALDI-MS analysis (Fig. 3), the purified protein contains full length PCT (Ala_1-Pro_{116}) with an additional Met residue at the N-terminus of the molecule and partially truncated PCT lacking the first alanine (Pro_2-Pro_{116}).

This recombinant PCT can be used as a calibrator in procalcitonin or calcitonin immunoassays.

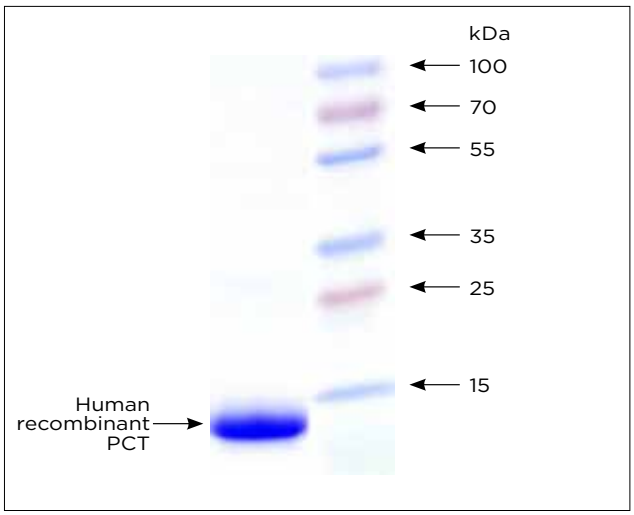


FIGURE 2. SDS-PAGE of purified human recombinant PCT (5 µg) in reducing conditions. Purity was determined by a densitometry analysis of the gel.

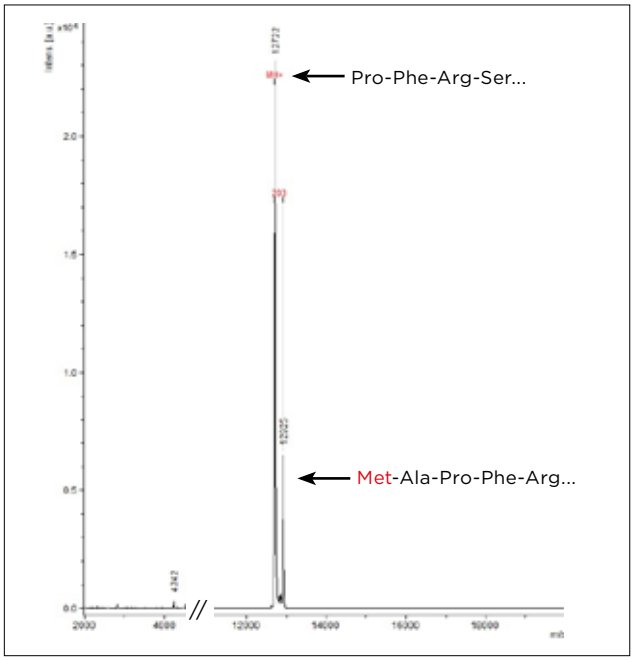


FIGURE 3. Mass spectrometry analysis of recombinant PCT

Stability studies

In order to find out how well our recombinant antigen retains its immunoreactivity after dissolving the lyophilized product in 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 (Fig. 4). It was also tested after repeated freeze-thaw cycles (Fig. 5). Our results show that the antigen is robust and retains its activity well under the tested conditions.

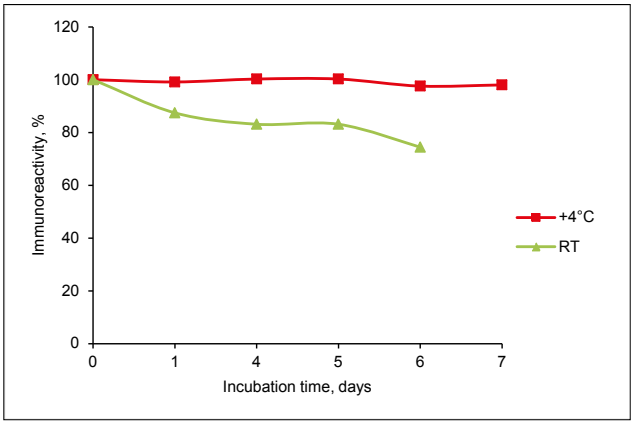


FIGURE 4. Stability of 1 mg/ml antigen solution at 4°C and room temperature (RT). PCT at 1 mg/ml concentration was incubated at +4°C or RT for the time periods indicated. Immunoreactivity was measured in sandwich ELISA with MAb pair 16B5-42.

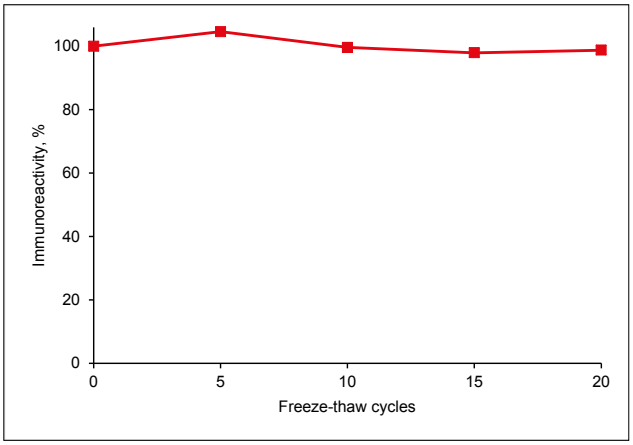


FIGURE 5. Stability of 1 mg/ml antigen solution after repeated freeze-thaw cycles. PCT at 1 mg/ml concentration was frozen at -70°C and thawed at RT for the indicated number of times. Immunoreactivity was measured in sandwich ELISA with mAb pair 16B5-42.

Comparison study

We compared our recombinant, tag-free PCT with a recombinant, tag-free PCT from another supplier. Our results show that there was no difference in immunoreactivity of these proteins (Fig. 6).

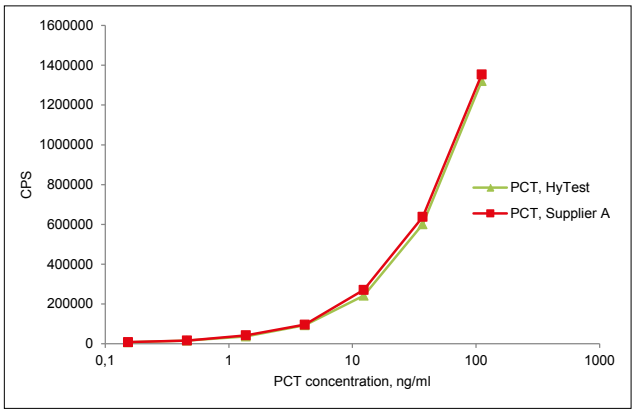


FIGURE 6. Comparative titration of tag-free PCT from HyTest and from supplier A. Immunoreactivity was measured in sandwich ELISA using mAb pair 16B5-42.

Monoclonal antibodies specific to PCT, calcitonin or katacalcin

Anti-PCT monoclonal antibodies

With our in-house immunoassay platform (DELFIA® immunoassay), the best sensitivity in terms of PCT detection was obtained using the following mAbs: mAb 16B5 (calcitonin-specific, capture) and mAb 42 (N-terminal PCT, detection) (Fig. 7). However, most of the mAbs could be used in different combinations and suitable combinations should be evaluated separately for different platforms.

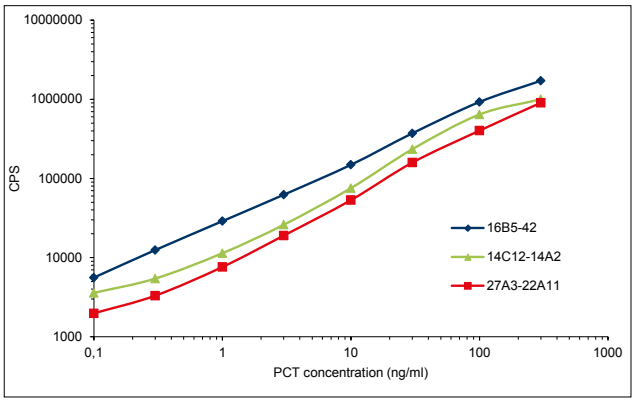


FIGURE 7. Calibration curves for three human PCT sandwich fluoroimmunoassays utilizing antibodies with different epitope specificity.
Capture mAbs: 1 µg/well
Detection mAbs (Eu^{3+}-labeled): 0.1 µg/well
Antigen: PCT human recombinant
Incubation time: 30 min

We also tested different 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. Serum titration curves for the assay 16B5 (capture) – 42 (detection) are shown in Fig. 8.

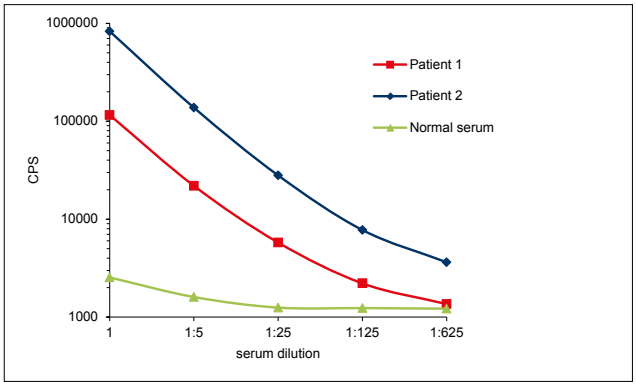


FIGURE 8. Titration of human serum samples from patients with sepsis of bacterial origin and one healthy individual (normal serum). Pair 16B5-42 (capture-detection) was used in a sandwich fluoroimmunoassay.
Capture mAb 16B5: 1 µg/well
Detection mAb 42 (Eu^{3+}-labeled): 0.1 µg/well
Incubation time: 45 min

Anti-calcitonin monoclonal antibodies

Calcitonin is a small peptide hormone that participates in calcium and phosphorus metabolism. Calcitonin is formed from PCT by posttranslational cleavages. Cleaved immature calcitonin is further processed into mature calcitonin by removal of the C-terminal glycine. Mature calcitonin is stored in secretory granules within the cells. Secretion of calcitonin is regulated by the level of Ca^{2+} in the blood. Mature calcitonin consists of 32 amino acid residues with a molecular weight of approximately 3.4 kDa, and theoretical pI 6.72.

We offer mAbs that are highly specific to different epitopes of the calcitonin molecule. Some of these antibodies are also recommended for PCT immunodetection when used in combination with N-terminal PCT or katacalcin specific antibodies. The titration curve of anti-calcitonin mAb 13B9 is shown in Fig. 9.

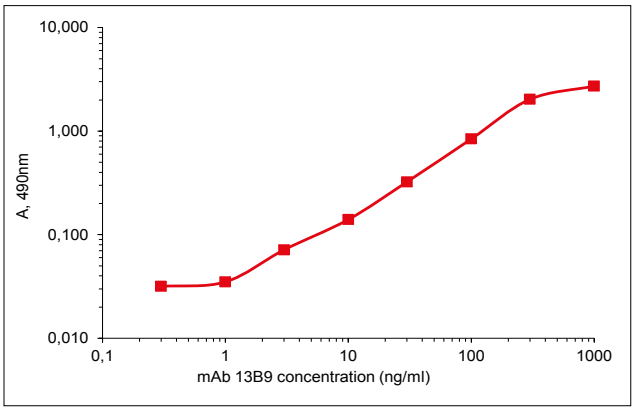


FIGURE 9. Titration curve of anti-Calcitonin mAb 13B9 in indirect ELISA. Antigen: Calcitonin human recombinant - 0.02 µg/well

Antibodies were also tested for their ability to detect PCT using Western blotting. All mAbs recognize PCT in Western blotting after SDS-electrophoresis in reducing conditions (Fig. 10).

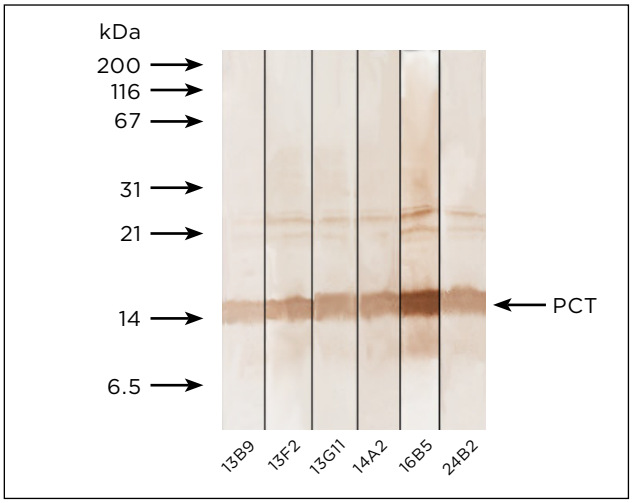


FIGURE 10. Detection of human recombinant PCT (100 ng/lane) by monoclonal antibodies specific to calcitonin in Western blotting after 15% SDS-PAGE in reducing conditions.

Anti-katacalcin monoclonal antibodies

Katacalcin is the C-terminal part of the procalcitonin molecule and it consists of 21 amino acid residues with a molecular weight of approximately 2.4 kDa, and theoretical pI 5.26. The physiological role of katacalcin is unknown. Antibodies that are specific to katacalcin could be used for the specific and sensitive detection of PCT in human blood. It is recommended to use antibodies recognizing katacalcin mostly as the capture antibodies in PCT immunoassays. The titration curve of anti-katacalcin mAb 14C12 is shown in Fig. 11.

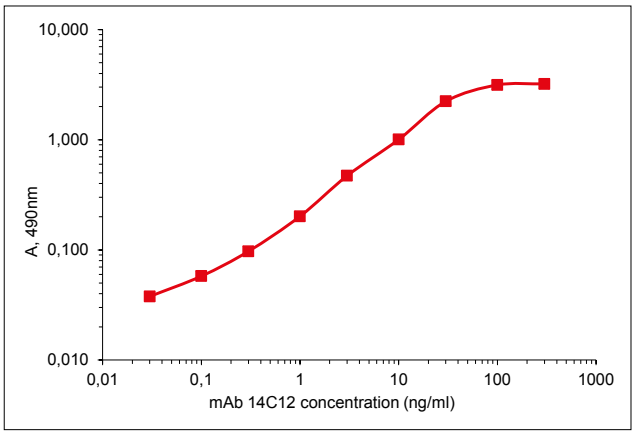


FIGURE 11. Titration curve of anti-katacalcin MAb 14C12 in indirect ELISA. Antigen: Katacalcin conjugated with BSA - 0.02 µg/well.

Antibodies were also tested for their ability to detect PCT in Western blotting. All mAbs recognize PCT in Western blotting after SDS-electrophoresis in reducing conditions (Fig. 12).

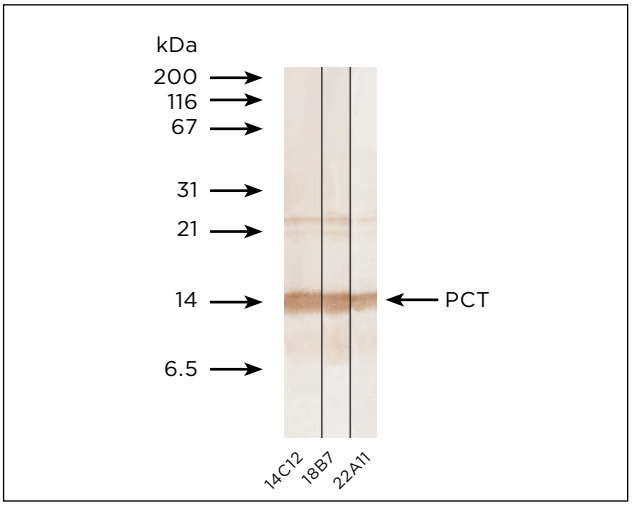


FIGURE 12. Detection of human recombinant PCT (100 ng/lane) by monoclonal antibodies specific to katacalcin in Western blotting after 15% SDS-PAGE in reducing conditions

Anti-N-terminal PCT monoclonal antibodies

The N-terminal fragment of procalcitonin is a peptide that consists of 57 amino acid residues. mAbs that are specific to N-terminal PCT are recommended for PCT immunodetection when used together with anti-calcitonin or anti-katacalcin antibodies. Anti-N-terminal PCT antibodies work better as the detection antibodies in a sandwich immunoassay. The titration curve for anti-N-terminal PCT mAb 42 is shown on Fig. 13.

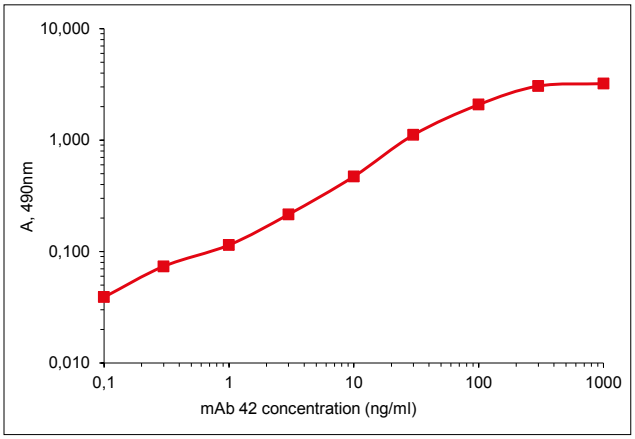


FIGURE 13. Titration curve of anti-N-terminal PCT MAb 42 in direct ELISA. N-terminal PCT conjugated with BSA - 0.02 µg/well.

Antibodies were also tested for their ability to detect PCT in Western blotting. All mAbs recognize PCT in Western blotting after SDS-electrophoresis in reducing conditions (Fig. 14).

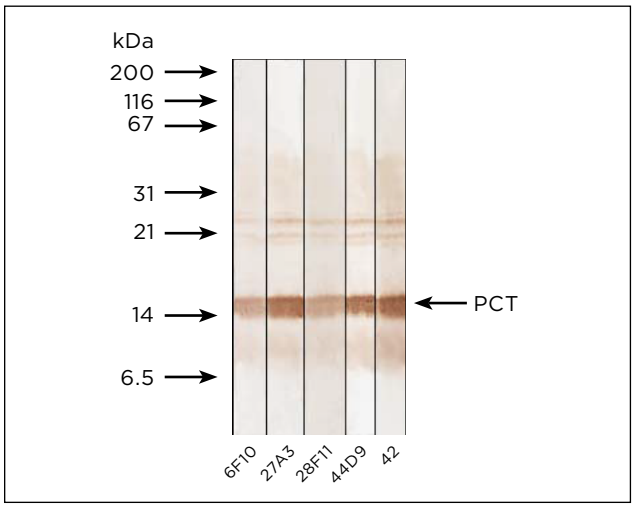


FIGURE 14. Detection of human recombinant PCT (100 ng/lane) by monoclonal antibodies specific to N-terminal fragment of PCT in Western blotting after 15% SDS-PAGE in reducing conditions.

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks	
Calcitonin	4C10	13B9	IgG2a	EIA, a.a.r. 60-69 of PCT	
		24B2	IgG1	EIA, WB, a.a.r. 72-81 of PCT	
		13F2	IgG1	EIA, WB, a.a.r. 72-81 of PCT	
		13G11	IgG1	EIA, WB, a.a.r. 72-81 of PCT	
		14A2	IgG1	EIA, WB, a.a.r. 72-81 of PCT	
		16B5	IgG2b	EIA, WB, a.a.r. 72-81 of PCT	
Calcitonin, *in vitro*	4C10cc	14A2cc	IgG1	EIA, WB, a.a.r. 72-81 of PCT	New!
Procalcitonin	4PC47	44D9	IgG2a	EIA, WB	
		6F10	IgG1	EIA, WB, a.a.r. 21-40 of PCT	
		27A3	IgG2a	EIA, WB, a.a.r. 21-40 of PCT	
		38F11	IgG1	EIA, WB, a.a.r. 21-40 of PCT	
		42	IgG2a	EIA, WB, a.a.r. 21-40 of PCT	
		22A11	IgG1	EIA, WB, a.a.r. 96-105 of PCT	
		14C12	IgG1	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	New!

ANTIGEN

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

References

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5. **Meisner M and Reinhart K** (2001) Is procalcitonin really a marker of sepsis? Int J Intensive Care 8(1), 15-25.

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