

Canine thyroid stimulating hormone (TSH, thyrotropin)



Hypothyroidism is one of the most common canine endocrine disorders (for review, see reference 1). It is a condition that is characterized by the deficiency of active thyroxine (T₄) and triiodothyronine (T₃) hormones which

are produced by the thyroid gland. In dogs, primary hypothyroidism is the most common form of the disease. It results from the destruction of the thyroid gland following lymphocytic thyroiditis or idiopathic atrophy.

In contrast to humans, it is often difficult to make a definitive diagnosis for dogs. The diagnosis of hypothyroidism is based on the presence of clinical signs, thyroid function test results and response to the thyroid hormone replacement therapy. Clinical signs are usually non-specific and indefinite often including lethargy, weight gain and various skin related problems. A low circulating total T₄ concentration suggests hypothyroidism. However, in order to reliably evaluate canine thyroid function, the results of the total T₄ measurement should be combined with measurements of free T₄ and TSH. Dogs with primary hypothyroidism would be expected to have low total T₄ and free T₄ levels and high TSH concentrations. Furthermore, anti-thyroglobulin antibodies can be screened as their presence is an indication of lymphocytic thyroiditis which may lead to hypothyroidism.

Biochemical properties of canine TSH

TSH belongs to the glycoprotein hormone family that also includes the luteinizing hormone (LH), the follicle stimulating hormone (FSH) and chorionic gonadotropin (hCG). Each of these hormones consists of two noncovalently linked subunits: Alpha and beta.

The alpha subunit is common for all four hormones of this group. There is a 73% sequence homology between human and canine alpha subunits. The canine alpha subunit consists of 96 amino acid residues (aar), which is four aar longer compared to the human alpha subunit. The calculated molecular weight of the canine alpha subunit derived from the amino acid sequence is 10,693 Da. Similarly to the human alpha subunit, the canine alpha subunit contains two potential N-glycosylation sites at residues 56 and 82 and five intramolecular disulfide bonds (2).

The beta subunits are hormone-specific. The beta subunits of canine and human TSH are highly homologous (91%). Both consist of 118 aar and contain six intramolecular disulfide bonds. The calculated molecular weight of the canine TSH beta subunit (protein part) is 13,517 Da. There is only one potential N-glycosylation site that is located at position 23 in both species. Two allelic variants in the canine beta subunit that differ at position 81 (Val81Ala) have been reported (3).

Monoclonal antibodies and recombinant canine TSH for the development of immunoassays

Advanced ImmunoChemical provides three monoclonal antibodies (MAbs) – 1CT1, 7CT8 and 11E4cc – that enable the development of a sensitive and specific canine TSH immunoassay. These MAbs have been raised against native human TSH and they recognize three different epitopes on the beta subunit of TSH. These MAbs recognize native canine TSH in canine serum and a recombinant canine TSH. The lack of cross-reactivity with other glycoprotein hormones was confirmed in cross-reaction studies with human LH, FSH, and hCG.

We also provide a recombinant canine TSH expressed in a mammalian cell line.

Monoclonal Antibodies

Development of a sandwich immunoassay for canine TSH measurements

Two MAb combinations (capture- detection) are recommended for the development of a sandwich immunoassay in order to measure TSH in canine serum samples: 11E4cc-1CT1 and 7CT8-1CT1. The calibration curves for the in-house immunoassays utilizing these MAb combinations are presented in Figure 1.

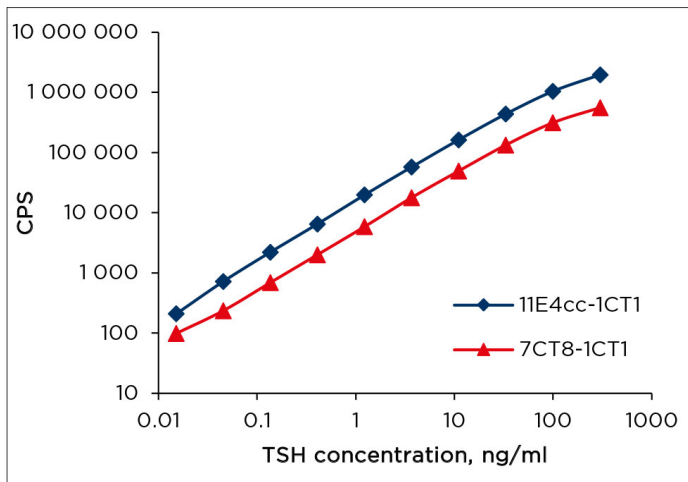


Figure 1. Calibration curves for immunoassays 11E4cc-1CT1 and 7CT8-1CT1. The immunoassays were performed in 96-well microplates coated with streptavidin (PerkinElmer). The capture antibodies 11E4cc and 7CT8 were conjugated with biotin. The detection antibody 1CT1 was conjugated with stable Eu³⁺ chelate. Recombinant canine TSH was used as a calibrator.

TSH concentration in the serum from dogs with excluded (n=10) or confirmed (n=13) hypothyroidism was measured using the two recommended antibody pairs. Median serum TSH concentrations were significantly higher in dogs with hypothyroidism compared with healthy dogs (see Figure 2).

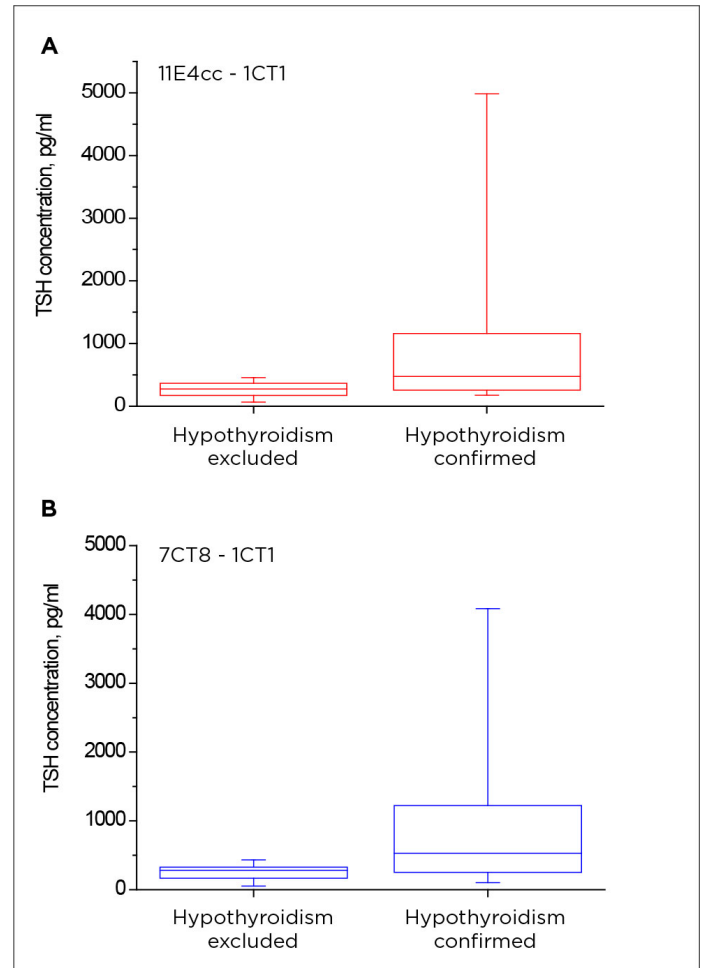


Figure 2. TSH concentration in the serum of healthy (n=10) and hypothyroid (n=13) dogs determined with the 11E4cc-1CT1 (A) and 7CT8-1CT1 (B) immunoassays. Results are displayed as a box-whisker plot. Horizontal lines indicate median values, boxes indicate values between the 25th and 75th percentiles, while whiskers indicate the minimum and maximum values.

ELISA

All of the MAbs recognize recombinant canine TSH in ELISA. A representative titration curve is provided for the MAb 1CT1 in Figure 3.

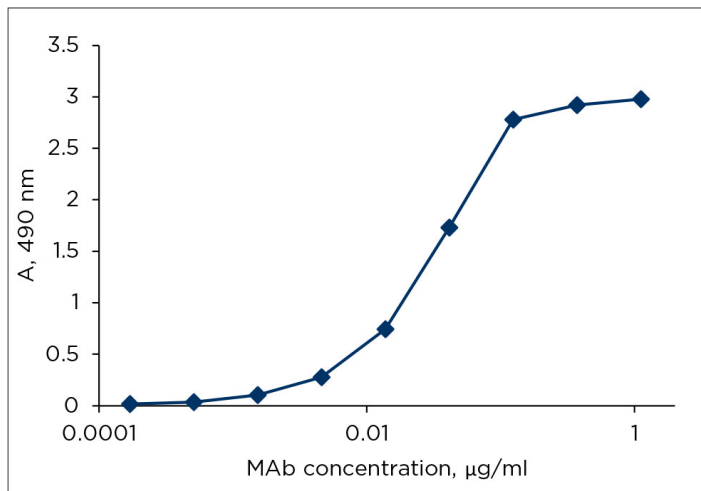


Figure 3. A representative titration curve of the MAb 1CT1 in ELISA. Recombinant canine TSH (0.03 µg/well) was used as an antigen.

Recombinant canine TSH

Recombinant canine TSH is produced by co-expression of the alpha (UniProt Q9XSW8) and beta (UniProt P54828) subunits of TSH in a mammalian cell line. Of the two known allelic variants the beta subunit of the recombinant canine TSH contains alanine at position 81. Neither of the subunits contain additional tags. The purity of the recombinant canine TSH product is > 90% and it might contain the free beta subunit.

The degree of recombinant canine TSH purity was analyzed by SDS gel electrophoresis in reducing conditions (see Figure 4, lane 1). The upper band on the gel corresponds to the alpha subunit and the lower band corresponds to the beta subunit. The subunits were identified by mass spectrometric analysis of protein bands isolated from a SDS-PAGE gel.

The electrophoretic mobility and relative positions of TSH subunit bands in the gel differ for electrophoresis in reducing and non-reducing conditions. In non-reducing conditions two major bands and one minor band were stained on the gel. TSH subunits were identified by Western blotting using a monoclonal antibody specific to the beta subunit (11E4cc) and a monoclonal antibody specific to human hCG (Cat.# 1-HCG1, the MAb 77F12) which demonstrates weak cross-reactivity with the alpha subunit of canine TSH. Two bands cor-

responding to canine TSH beta subunit and a single band corresponding to canine alpha subunit were detected in Western blotting studies (see Figure 5).

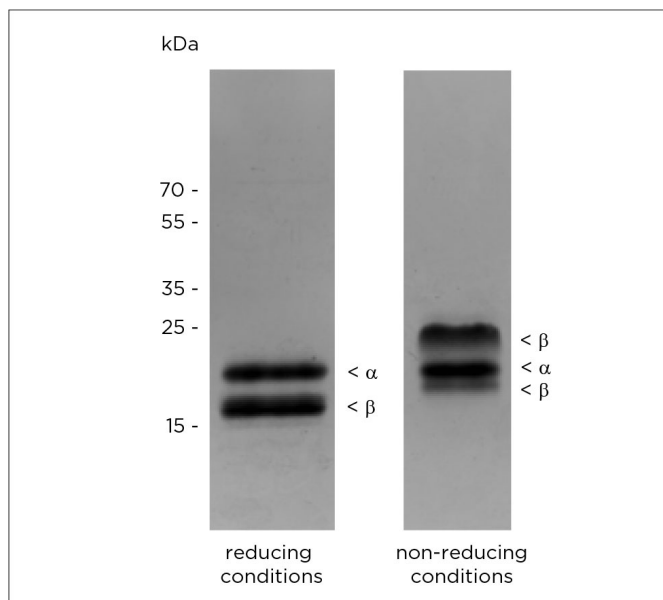


Figure 4. SDS gel electrophoresis of recombinant canine TSH. 4 µg of purified TSH was analyzed by SDS gel electrophoresis in 10-20% gradient gel in reducing (lane 1) and non-reducing (lane 2) conditions. Protein bands were visualized by Coomassie Blue (R- 250) gel staining.

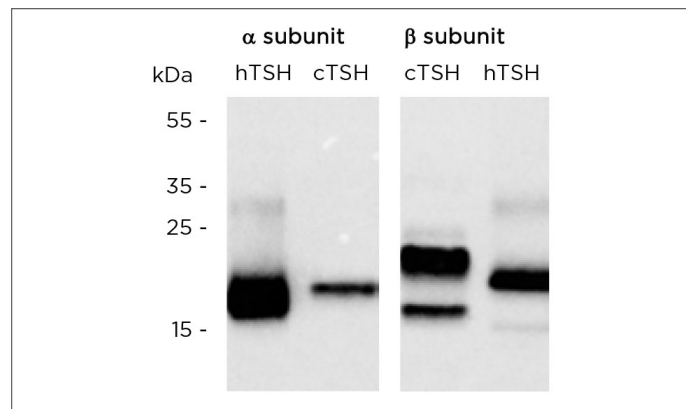


Figure 5. Detection of alpha and beta subunits of canine TSH in Western blotting. Recombinant canine TSH (cTSH) and native human TSH (hTSH; used as a standard), 1 µg per lane, were applied to 10-20% gradient SDS-PAGE gel, run in non-reducing conditions and transferred onto nitrocellulose membrane. Alpha subunit lanes were stained using monoclonal antibody detecting the alpha subunit (Cat.# 2H8, MAb 77F12). Beta subunit lanes were stained using monoclonal antibody specific to the beta subunit (11E4cc).

Ordering Information:

MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
Thyroid stimulating hormone (TSH)	1-TSH	1CT1	IgG1	EIA, WB in non-reducing conditions
		7CT8	IgG1	EIA
Thyroid stimulating hormone (TSH), in vitro	1-TSHiv	11E4cc	IgG1	EIA, WB in non-reducing conditions

ANTIGENS

Product Name	Cat. #	Purity	Source
Recombinant canine thyroid-stimulating hormone (TSH)	8-TSH-c	>90%	Mammalian cell line

References

1. **Mooney CT.** Canine hypothyroidism: a review of aetiology and diagnosis. *N Z Vet J.* 2011 May;59(3):105-14.
2. **Yang X, McGraw RA, Ferguson DC.** cDNA cloning of canine common alpha gene and its co-expression with canine thyrotropin beta gene in baculovirus expression system. *Domest Anim Endocrinol.* 2000 May;18(4):379-93.
3. **Yang X, et al.** Canine thyrotropin beta-subunit gene: cloning and expression in *Escherichia coli*, generation of monoclonal antibodies, and transient expression in the Chinese hamster ovary cells. *Domest Anim Endocrinol.* 2000 May;18(4):363-78.

