Fact sheet of monoclonal antibodies (mAbs) against human chorionic gonadotropin (hCG) & related variants

hCG Function

Physiologically, human chorionic gonadotropin (hCG) and related molecules are secreted by the syncytiotrophoblast from day 5 after conception and are essential for maintaining pregnancy. They do so by stimulation of secretion of progesterone from corpus luteum, which in turn helps building up endometrial mucosa, prevents menstrual bleeding and –via hypophyseal negative feedback- inhibits further ovulation. Due to these properties, hCG molecules have an important diagnostic role in detection of pregnancy from peripheral blood. In addition, hCG and hCG-related molecules are produced in a variety of tumors that can be identified through hCG measurement (1) (2).

hCG Molecular Structure and Immunogenic Epitopes

hCG belongs to the glycoprotein hormone family like follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH). It is a protein heterodimer consisting of two subunits (hCGα and hCGβ) which are stabilized by three disulfide bonds forming a cysteine knot. These two subunits together form the biologically active αβ-heterodimer termed hCG. Common to all these glycoprotein hormones is the α-subunit encoded by a single gene, while the β-subunit is hormone specific.
Preferentially, the mAbs against hCG and related variants recognize epitopes determined by the tertiary or quaternary structure of hCG (1). The epitopes located on hCG and hCG-related molecules are as follows (1):

- Intact hCG αβ-heterodimer (holo hormone)
    - 4 conformationally determined epitopes (c1-c4), not found on free hCG subunit
- hCG α subunit: 7 epitopes (α1-α7)
    - Epitopes α6 and α7 are specific for the non-assembled hCG α.
- hCG β subunit: 9 epitopes (β1-β9)
- hCG βcf: 4 epitopes (β10-β13)
    - Epitopes β6 and β7 are shared only by free hCGβ and hCGβcf.
    - The specific hCG βcf epitopes are not present on hCG β, hCG βn, hCG, hCG n, or on hLH/hLHβ/hLHβcf (2).
    - HCGβcf is a major urinary metabolite in pregnant women and cancer patients with tumours producing hCG and is absent in serum. This metabolite (amino acids 6-40 linked to 55-92 *via* five disulfide bonds) contains the immunodominant structure of hCG and accommodates 4 specific hCGβcf determinants (β10-β13). Those determinants are not found on hCG or intact hCGβ subunit (1) (3).

It is of major importance to use antibodies with appropriate specificities for hCG, hCG variants and other glycoprotein hormones when designing hCG methods to detect specific diseases (**Figure 1**) (3) (4).

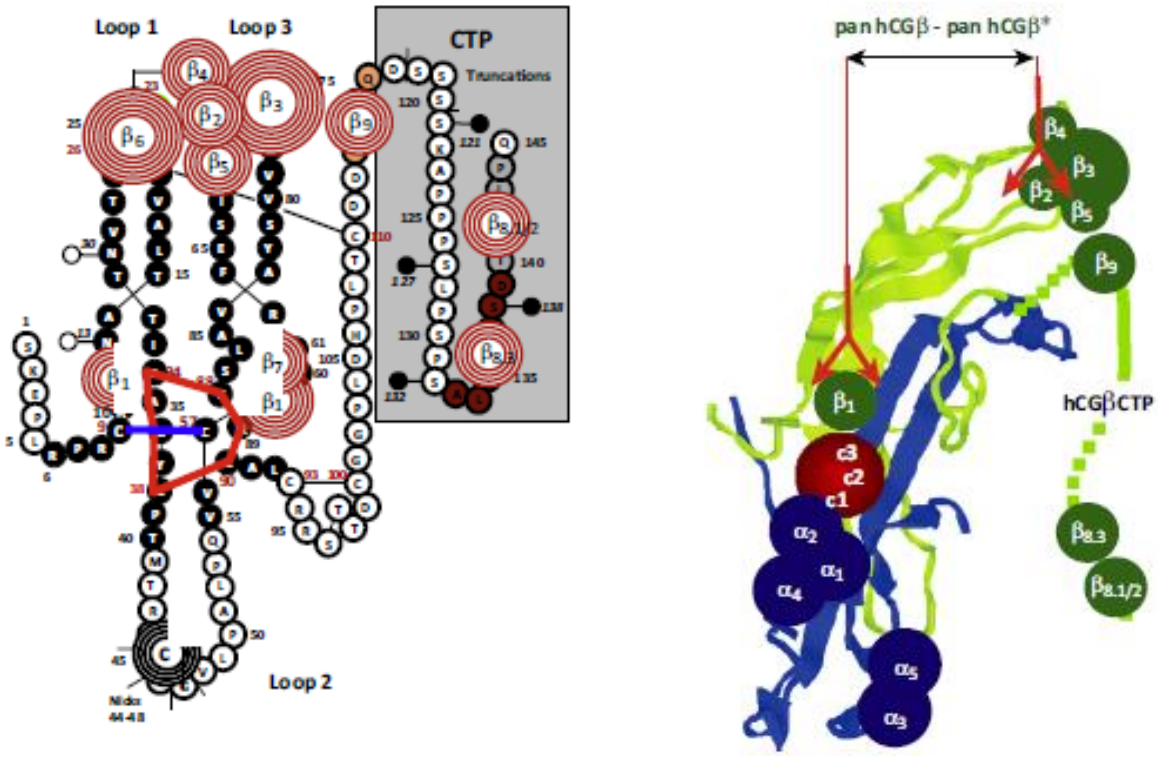

Figure 1. Left: 3D hCGβ map showing antigenic domains/epitopes of hCG. Right: 3D epitope map of hCG and location of epitopes b1–b2/4 for the construction of a wide spectrum of methods for the detection of hCG+hCGβ+hCGβcf methods

The mAbs of Wick Diagnostics' portfolio recognize most of these epitopes and therefore are useful for the construction of selective immunoassays.

hCG Monoclonal Antibodies Available at Wick Diagnostics GmbH

Quality initiatives

- The purpose of the first and second **ISOBM TD-7 workshops** (International Society of Oncology and Biomarkers) was to harmonize the comparability for measurement of the heterogeneous hCG molecules in immunoassays. For these studies among others our well characterized monoclonal antibodies served as references for epitope mapping studies of diagnostic antibodies of unknown epitope recognition by the ISOBM TD-7 (3). Specificity and epitopes recognized by the reference mAbs produced in Innsbruck (INN-) are listed in **Table 1** (1) (2).
- Using among others our reagents the well respected non-profit organization **UK NEQAS** (United Kingdom National External Quality Assessment Service) performed recovery experiments for quantitative measurements of hCG. These showed that there is a higher variation coefficient between hCG measurement in pregnancy urines than in specimens containing patient sera. This may reflect different concentrations of hCGβcf in urine, while high concentrations of hCG and hCG variants can cause a 'hook-effect', leading to false lower results. It was thus learnt that to achieve better results in pregnancy tests from urine the antibody selection and standardisation of antigenic domains and epitopes have to be considered

Technical notes

Our mAbs are obtained from a great number of fusion experiments and are well characterized. The ascites-derived mAbs were purified by salt precipitation and HPCL, dialysed against PBS and lyophilized.
They can be used in immunoradiometric assay (IRMA), immunoenzymometric assay (IEMA) and time-resolved fluoroimmunoassay (IFMA) according to their epitope recognition either as capture or detection antibodies in one-sided or sandwich immunoassays. Phosphate buffered saline (PBS) should be used as coating buffer. Coating should take place at +4° degree overnight. When used as a detection antibody, labelling with iodine ^{125}, horseradish peroxidase (HRPO) or Europium (Eu^{+}) is possible. For blocking buffer we recommend 1 %BSA in PBS containing 0,01 % Thiomersal. The washing buffer consists of PBS containing 0,05 % Tween 20 and 1 % Thiomersal for preservation.
Lyophilized antibodies should be stored at +4°C. To avoid freezing and thawing the resuspended mAbs should be stored in aliquots at – 20°C. For long term storage -20° to -90° degree is necessary.

Clinical diagnostic use

The mABs in their current format are for research use only (RUO), apt to be transferred into product development. Intended uses could be:

- Early pregnancy monitoring
- Prenatal screening, e.g. Down Syndrome
- Tumor diagnosis (testes, ovaries, placenta)

For construction of immunoassays for hCG and /or hCG variants we recommend the following mAb combinations (Table 1).

Specificity	MAb combination	Possible clinical applications
hCG + hCGβ-like-molecules	β1 mAb (e.g. INN-hCG-2) – β2 or β4 detection mAb (e.g. INN.hCG-22)	Oncology Early pregnancy monitoring Prenatal screening (primarily Down´s)
hCG + hCGn	c3 mAb (e.g. INN_hCG-45) - β2 or β4 detection mAb (e.g. INN-hCG-22)	Early pregnancy monitoring Prenatal screening (primarily Down´s) Oncology, provided hCGβ is also measured in an second assay
hCG	c1/2 mAb - β2 or β4 detection mAb (e.g. INN-hCG-22)	Early pregnancy monitoring Prenatal screening (primarily Down´s)
hCGβ+hCGβcf	β6/7 mAb (e.g. INN-hCG-68) - β2 or β4 detection mAb (e.g. INN-hCG-22)	Prenatal screening (primarily Down´s) Oncology, provided hCG+hCGn is also measured in an second assay
hCGβcf only	β11 mAb (e.g. INN-hCG-106) - β2 or β4 detection mAb (e.g. INN-hCG-22)	Urine measurements only Clinical utility still to be established (possibly nontrophoblastic tumors)
hCGα only	α6 mAb (e.g. INN-hCG-72) – α5 detection mAb (e.g. INN-hFSH-158)	Pituitary tumors secreting α-subunit Testicular cancer

Table 1. Selection of combinations for sandwich immunoassays to detect hCG in various indications.

Portfolio of mAbs at Wick Diagnostics

Wick Diagnostics offers a variety of monoclonal antibodies of highest quality, which detect glycoprotein hormones produced by the human anterior hypophysis:

Table 2. hCG reference mAbs (modified according to (1)).

mAb-Code INN-	Epitope	hCG	hCGβ	hCGβcf	hCGn	hCGβn	hLH	hLHβ	hFSH hTSH	GPHα
hCG-2	β_1	■	■	■	■	■				
hCG- 22	β_2	■	■	■	■	■				
bLH-1	β_3	■	■	■	■	■	■	■		
hCG- 24	β_4	■	■	■	■	■				
hCG- 58	β_5	■	■	■	■	■	■	■		
hCG- 64	β_6		■	■						
hCG- 68	β_7		■	■						
hCG- 103	β_{10}			■						
hCG- 104 hCG- 106	β_{11}			■						
hCG- 105	β_{12}			■						
hCG- 112	β_{13}			■						
hFSH- 73	α_1	■			■		■		■	■
hFSH- 98 hFSH- 100	α_2	■			■		■		■	■
hFSH- 132	α_3	■			■		■		■	■
hFSH- 179	α_4	■			■		■		■	■
hFSH- 158	α_5	■			■		■		■	■
hCG- 72 hCG- 80	α_6									■
hCG- 10	c_1	■								
hCG- 40 hCG- 53	c_2	■								
hCG- 45	c_3	■			■					
hCG- 26	c_4	■			■		■			

GPHα = glycoprotein hormone alpha subunit; n = nicked; βcf = β-core fragment; h = human; LH = luteinizing hormone; LHβ = luteinizing hormone subunit; FSH = follicle-stimulating hormone; TSH = thyroid-stimulating hormone. Filled squares = strong reactivity; white squares = no reactivity

Literature

1. **Berger, P, et al.** The ISOBM TD-7 Workshop on hCG and Related Molecules. *Tumor Biology.* 2002, pp. 23:1-38.

2. **Berger, P., et al.** Candidate epitopes for measurement of hCG and related molecules: the second ISOBM TD-7 workshop. *Tumor Biology.* 2013, pp. 34: 4033-4057.

3. **Berger, P. and Sturgeon, C.** Pregnancy testing with hCG - future prospects. *Trends in Endocrinology and Metabolism .* 2014, Vol. 25, 12.

4. **A.J., Berger P. and Lapthorn.** Standardization of Epitopes for Human Chorionic Gonadotropin /hCG) Immunoassays. *Current Medical Chemistry .* 2016, 23.

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