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N.B.: When citing this work, cite the original article.

Original Publication:

Tove Sivik, Svante Vikingsson, Henrik Gréen and Agneta Jansson, Expression Patterns of 17β-Hydroxysteroid Dehydrogenase 14 in Human Tissues, 2012, Hormone and Metabolic Research, (44), 13, 949-956.
http://dx.doi.org/10.1055/s-0032-1321815
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http://www.thieme.com/

Postprint available at: Linköping University Electronic Press
http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-84681

Expression patterns of 17β hydroxysteroid dehydrogenase 14 in human tissues

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Running Head: Systemic distribution and activity of 17βHSD14

Abstract

17βHSD enzymes catalyse the stereospecific oxidation/reduction at carbon 17β of androgens and oestrogens, and are important players in intracrine sex hormone synthesis. The biological relevance of 17βHSD14, first named retSDR3, is largely unknown. We generated and validated an antibody targeting the 17βHSD14 antigen and used this for immunohistochemical evaluation of expression patterns in 33 healthy human tissues. Furthermore, sex steroid conversional activity in *HSD17B14* over-expressing HEK293 and MCF10A cells was investigated by assessing interconversion products of oestrone, oestradiol, androstenedione, testosterone and dehydroepiandrosterone. Immunohistochemical staining patterns of 17βHSD14 with the enzyme being primarily expressed in glandular epithelial tissue reveal an enzyme with possible implications in the secretion or conversion of externally derived compounds. A role for 17βHSD14 in sex steroid metabolism is supported by the finding that 17HSD14 oxidises both oestradiol and testosterone into less bioactive steroid metabolites oestrone and androstenedione respectively.

Key words: 17βHSD, immunohistochemistry, radio-HPLC

1 **1. INTRODUCTION**

2 The gene product of *DHRS10,* initially named retSDR3, and later 17β hydroxysteroid dehydrogenase
3 (17βHSD) 14, was cloned from a retinal epithelium cDNA-library in the year 2000 [1]. Based on
4 sequence analysis, the protein was determined to be a short-chain dehydrogenase/reductase (SDR),
5 and suggested to be involved in retinol metabolism; however such functional properties could not be
6 shown. In 2006, Lukacik and colleagues further investigated the *DHRS10* gene product [2]. Based on
7 structural and functional studies which revealed features of the protein equivalent to those of
8 17βHSDs, the *DHRS10* gene product was denoted 17βHSD14 [2].

9 Using the oxidised or reduced forms of NAD(P)(H) as hydrogen donors or acceptors, 17βHSD
10 enzymes catalyse the stereospecific oxidation/reduction at carbon 17β of androgens and oestrogens
11 [3]. Upon receptor binding, the 17β-hydroxy conformation of androgens and estrogens triggers a
12 greater biological response than the corresponding keto-conformation of the steroids [4], and the
13 17βHSD enzymes are thus important mediators in pre-receptor regulation of sex hormone action. Of
14 the 15 types described to date, 14 are found in human, and structurally, all but 17βHSD type 5 which
15 is an aldo-keto reductase, belong to the SDR-family [5,6]. The 17βHSD enzymes are a diverse protein
16 family, varying in tissue distribution as well as in substrate preferences. While the major substrates of
17 17βHSD enzymes are sex steroids [2,7-11], a few are believed to be dedicated primarily to other
18 substrates, such as fatty acids [12,13], cholesterols [14] bile acids [15] or retinoids [16]. Several of
19 the 17βHSDs can work essentially as both reducing and oxidising agents depending on cofactor
20 concentrations and pH, yet *in vivo* they tend to be unidirectional, and according to experimental data
21 the 17βHSD enzymes are grouped as either oxidative or reductive enzymes based on preferred co-
22 factor utilization under biological conditions. From this classification, human 17βHSD types 2, 4, 6, 8,
23 10, 11 and 14 are considered *in-vivo* oxidative enzymes catalyzing the NAD(P)+-dependent
24 inactivation of oestrogens/androgens whereas types 1, 3, 5, 7, 12 and 15 catalyze the NAD(P)H-
25 dependent reduction, and hence activation, of oestrogens and androgens [5,6].

26 By catalyzing the final steps in steroid metabolism, the 17βHSD enzymes, alongside enzymes such as
27 3β-hydroxysteroid dehydrogenases, aromatase and sulfotransferases/sulfatases, are thought to be

1 important contributors in intracrinology. This element in endocrinology deals with peripheral sex
2 steroid synthesis from adrenally derived precursors, e.g. dehydroepiandrosterone (DHEA), into more
3 potent metabolites such as estradiol (E2) and testosterone (T) [17]. In intracrinology, the biological
4 effect of a sex steroid is the result of local synthesis rather than an outcome mediated by circulating
5 active sex steroids derived from endocrine organs, i. e. the gonads. The local distribution and
6 expression levels of oxidative versus reductive steroidogenic enzymes will eventually affect the fine-
7 tuning of hormonal profiles in a given target tissue or cell type. In addition to significant roles in
8 normal physiology, many of the enzymes involved in intracrine sex-steroid synthesis have been
9 implicated in steroid-associated pathological conditions such as breast and prostate cancer [18,19].

10 The current study further elucidates the biological role of 17βHSD14. An antibody targeting
11 17βHSD14 was prepared and used for determining expression patterns in 33 human tissues. Moreover,
12 the enzymatic potential in converting E2, oestrone (E1), androstenedione (A) and T, was assessed in
13 media from cells transiently over-expressing *HSD17B14.*

14

15 ## 2. MATERIAL AND METHODS

16 ### *2.1 Preparation of polyclonal anti-17βHSD14 antibody*

17 The peptide sequence (NH2-)CKASRSTPVDAPDIP(-CONH2) corresponding to amino acids 255 to
18 269 of human 17βHSD14 with an additional terminal cystein residue for affinity purification purposes,
19 was synthesised and sequenced by AgriSera (Vännäs, Sweden). A breed of New Zeeland white
20 rabbits/French lop received initial subcutaneous injections of 200 µg peptide dissolved in a buffer
21 emulsified in Freunds Complete Adjuvant. Three booster injections were administered at 3 months
22 interval; the first including 200 µg peptide and the two following 100 µg. Freunds Incomplete
23 Adjuvant was used with the booster injections. The animals were finally bled one week after the last
24 immunisation and were thereafter sacrificed by injection of an intravenous pentobarbital (Apoteket
25 Stockholm, Sweden). The anti-17βHSD14 antibody was affinity-purified on a column containing a
26 peptide-coated gel matrix (Ultralink; Thermo Fischer Scientific, Waltham, MA). The preparation of

1 the 17βHSD14 antibody was performed with permission given by the Swedish animal welfare
2 authority (dnr A112-06). Peptide-neutralisation assay as well as immunoblot analysis of *HSD17B14*-
3 transfected HEK293- and MCF10A cells confirmed antibody specificity.
4
5 ***2.2 Immunohistochemical evaluation of 17βHSD14 expression in various human tissues***
6 Paraffin-embedded tissue micro array (TMA)-slides composed of totally 33 human tissues from
7 healthy individuals including adrenal gland (n=3), myeloid bone (n=8), breast (n=60), brain (n=14),
8 colon (n=60), eye (n=3), esophagus (n=8), heart (n=3), kidney (n=8), larynx (n=3), liver (n=8), lung
9 (n=8), lymph node (n=5), mesothelium (n=3), nerve (n=3), ovary (n=8), pancreas n= 8), parathyroid
10 gland (n=3), pituitary (n=3), prostate (n=60), rectum (n=5), salivary gland (n=3), skeletal muscle
11 (n=3), skin (n=8), duodenum (n=5), spleen (n=8), stomach (n=8), testis (n=8), thymus (n=8), thyroid
12 gland (n=3), tonsil (n=3), uterine cervix (n=8), uterus (n=3), all in 1 mm diameter cores of 5 μm
13 thickness were purchased from Tissue Array Network (Rockville, MD). TMA slides were
14 deparaffinised in xylene and then hydrated in descending concentrations of ethanol. The sections were
15 thereafter treated in a decloaking buffer (Biocare Medical, Concord, CA) in a decloaking chamber to
16 retrieve antigenicity. The temperature was allowed to reach 120°C at which the heat was turned off.
17 To reduce non-specific staining, sections were immersed in phosphate-buffered saline (PBS)
18 supplemented with 0.1 % bovine serum albumin (BSA) and then incubated with a commercial protein
19 blocking solution (Spring Bioscience, Pleasanton, CA) for 10 minutes. The tissue sections were
20 incubated with the in-house polyclonal rabbit anti-human 17βHSD14 antibody diluted to 1:1200 in
21 antibody diluent (DakoCytomation, Glostrup, Denmark) at 4°C over night. After a washing step in
22 PBS/BSA, sections were incubated with EnVision horseradish peroxidate-conjugated anti-rabbit
23 polymer (DakoCytomation) for 25 minutes. The immune reaction was visualised by incubating the
24 samples in a solution containing 3,3-diaminobenzidine chromogen supplemented with hydrogen
25 peroxide for eight minutes. Sections were briefly counterstained with hematoxylin, dehydrated in
26 ascending concentrations of ethanol and finally mounted. A test for antibody specificity was
27 performed by peptide neutralising assay. Pre-incubation of antibody and 17βHSD14-peptide was done

1 for two hours in RT with peptide (10^{-6} M) and 17βHSD14 antibody (1:600) at a molar ratio of 100:1 in
2 tris-buffered saline with tween (TBST) without blocking reagent. The pre-incubation solution was
3 mixed with an equal volume of TBST supplemented with blocking solution and added to the TMA
4 tissue for overnight incubation. The TMAs were investigated microscopically using a Leica LB30T
5 microscope (Leica Microsystems, Wetzlar, Germany) by two independent investigators blinded to
6 clinical data (T.S and A.J), and the findings were confirmed by a pathologist (HO). Representative
7 slides were photographed using an Olympus SC20 digital camera (Olympus Europe GmbH, Hamburg,
8 Germany).

9

10 ***2.3 Transient transfection of cultured cells with HSD17B14***

11 HEK293 cells (American Type Culture Collection, ATCC, Manassas, VA; ATCC number CRL-1573)
12 were seeded at 16 000 cells/cm^2 in 24-well plates in 0.5 ml/well OPTIMEM phenol-red free Opti-
13 MEM medium (Invitrogen, Carlsbad CA) supplemented with 4% charcoal-treated serum (CTS)
14 (Invitrogen). MCF10A cells (ATCC number CRL-10781), were seeded at at 16 000 cells/cm^2 in 24-
15 well plates in 0.5 ml/well in ATCC complete growth medium supplemented with 20 ng/ml epidermal
16 growth factor, 100 ng/ml cholera toxin, 0.01 mg/ml insulin and 500 ng/ml hydrocortisone. All cells
17 were cultured at 37°C in 5% CO_2. Twenty-four hours after seeding, the cells were transfected with a
18 commercial *HSD17B14*-plasmid (Origene, Rockville, MD, USA), using Xtremegene transfection
19 reagent (Roche Diagnostics, Indianapolis, IN). Mock-transfected cells were incubated with the same
20 amount of a vector missing the *HSD17B14* insert. (Roche Diagnostics, Indianapolis, IN). Transfection
21 efficiency was evaluated by measuring 17βHSD14 expression in transfected cells with western blot.

22

23 ***2.4 Western blot***

24 30 µg of protein from lysates of non-transfected, mock-transfected cells was subjected to
25 electrophoretic separation on 5-15 % SDS-PAGE gels (BioRad, Hercules, CA) at 200 V for 30
26 minutes. The proteins were then blotted onto a PVDF-membranes for 60 minutes at 100 V.

1 Electrophoresis and western blotting was performed using the Criterion precast gel System (BioRad).
2 The membranes with blotted proteins were blocked in TBS-tween (TBST) supplemented with 5 %
3 non-fat skimmed milk (BioRad) for 60 minutes on a shaker after which it was incubated with an in-
4 house polyclonal rabbit anti-17βHSD14 antibody, diluted in blocking solution to 1:1000 at 4°C over
5 night. The membranes were then washed three times with TBST solution and incubated at ambient
6 temperature for 60 minutes with a secondary HRP-conjugated anti-rabbit IgG (DakoCytomation),
7 diluted to 1:2000 in blocking solution. The blot was washed three times in TBST and bound antibodies
8 were detected using ECL plus detection reagents (Amersham, Arlington Heights, IL). Results were
9 visualised using LAS1000 CCD-camera detection system (FujiFilm, Tokyo, Japan). A monoclonal
10 anti human β-aktin antibody (Cell Signaling, Beverly, MA) was used to control for equal loading.

11

12 ***2.5 Assessment of enzymatic conversion of tritiated substrates in cultured cells over-expressing***
13 ***HSD17B14***

14 Twenty four hours after transfection, cell culture media was aspired and replaced by Opti-MEM I with
15 4 % CTS supplemented with 10 nM of tritiated sex steroids; androst-4-ene-3, [1,2,6,7-^{3}H(N)]-, 17-
16 Dione (98.1 Ci/mmol; range 70-110 Ci), oestradiol, [2,4,6,7-^{3}H(N)]- (70 Ci/mmol; range 70-115 Ci),
17 oestrone, [2,4,6,7-^{3}H(N)]- (89.3 Ci/mmol; range 50-100 Ci), testosterone, [1,2,6,7-^{3}H(N)]- (70
18 Ci/mmol; range 70-105 Ci) and dehydroepiandrosterone, [1,2,6,7-^{3}H(N)]- (50 Ci/mmol; range 60-100
19 Ci) (Perkin Elmer, Waltham, MA. Cell culture media was collected at 0, 4, 8, 24, 48 and 72 hours, and
20 stored at -70°C until analysis. Experiments were run in duplicates and the whole procedure was
21 repeated at least three times. Media samples with tritiated sex steroid metabolites were incubated
22 without cells in order to monitor spontaneous steroid degradation within the given time interval.

23

24 ***2.6 Sample preparation and HPLC-analysis of steroid interconversion***
25 The procedure for sample preparation, separation and detection of sex steroid interconversion product
26 has been published elsewhere [20] and was used with minor modifications. Briefly, cell culture media

samples containing tritiated steroid interconversion products were precipitated using cold zinc sulphate solution (10%) and 1 M sodium hydroxide solution. After centrifugation, supernatants were transferred to injection vials for analysis. Samples were injected onto a Beckman Coulter Ultrasphere ODS column, 250 x 4.6 mm, 5 µm (Bromma, Sweden), maintained at 40°C. The retention time for androstenediol was experimentally determined using UV detection of unlabelled substrate. During the run the autosampler was kept at 15°C. Between two and 14 minutes scintillation fluid (Ultima-Flo, Perkin Elmer) was mixed with the column eluent in a 2:1 ratio enabling radiochemical detection of the tritium labeled steroids.

3. RESULTS

3.1 Antibody specificity

Immunoblot analysis of lysates from HEK293 and MCF10A cells transiently transfected with *HSD17B14* revealed a single band at expected size 28 kDa. Weak or no bands were present in mock-transfected or non-transfected cells (Fig. 1). When neutralized by a 17βHSD14-peptide, immunopositivity in liver was not evident (Fig. 3).

3.2 17βHSD14 distribution in human tissues

In order to evaluate the localisation of 17βHSD14 on a sub-tissue level we generated an antibody towards the antigen and used this for immunohistochemical analysis of the protein in 33 human tissues (Table 1). In specimens representing female reproductive organs (Fig. 2 A-D), uterus showed weak to moderate cytoplasmic immunoreactivity in the secretory glandular epithelium of the endometrium, whereas peritubular area, comprised of mainly smooth muscle, was unstained. Ovarian specimens showed immunoreactivity in granulosa cells of dominant follicles as well as in atretic corpus luteum. Breast specimens showed moderate to strong staining of 17βHSD14 in the cytoplasms of ductal epithelial cells. Periductal stromal areas were unstained. In male reproductive organs (Fig. 2 E and F),

1 prostate samples showed negative to weak immunoreactivity. In testis, immunoreactivity was seen in
2 most of the seminiferous epithelium as well as in peritubular areas harbouring Leydig cells.
3 17βHSD14 was highly expressed in kidney, with samples showing strong immunoreactivity in
4 glandular cells of both proximal and distal tubules while cells of Bowman's capsule and endothelial
5 cells of glomeruli remained unstained (Fig. 3 A). In retina, 17βHSD14 was present in cytoplasmic
6 projections of the plexiform layers whereas nuclear layers were negative, with an exception for the
7 inner nuclear layer, where staining was prominent in some nuclei (Fig. 3 B). 17βHSD14 expression
8 was also evident in the gastrointestinal (GI) area (Fig. 3 C-F); in stomach samples, staining was
9 relatively strong in upper mucosal areas whereas underlying stroma was unstained. Most 17βHSD14
10 appeared to be located cytoplasmically although some nuclear staining was evident. In duodenal
11 specimens, staining was weak to moderate throughout the mucosal layer. In colon and rectal samples,
12 the presence of 17βHSD14 was especially marked at the absorptive edges of the mucosa facing the GI-
13 lumen. Both enterocytes and goblet cells of the mucosal crypts stained, and although staining was
14 mostly cytoplasmic, as in the stomach, some nuclear staining was evident. In the deeper layers of the
15 mucosa, staining diminished gradually and was virtually absent below the inner muscular layer. When
16 tissues of both sexes were available, no apparent differences in staining intensities or distribution
17 patterns were seen when taking gender of tissue donor into account.

18

19 ***3.3 Steroid interconversion***

20 In order to assess steroid conversion by 17βHSD14, HEK293 cells were transfected with an
21 expression vector containing the *HSD17B14* insert. Transfected cells were thereafter exposed to
22 radiolabelled steroids, and generated tritiated products were separated by HPLC and detected by β-
23 scintillation. The rate of steroid interconversion of oestrone/oestradiol and
24 androstenedione/testosterone was obtained by integrating generated peak areas and computing ratios
25 of E1/E2 and A/T. *HSD17B14* transfected cells oxidised oestradiol and testosterone to oestrone and
26 androstenedione respectively with net efficiencies of approximately 15% for the oestradiol to oestrone
27 reaction and 8% for the testosterone to androstenedione conversion compared with mock-transfected

cells. No reduction of E1, A or DHEA exceeding that of mock-transfected cells was seen for *HSD17B14*-transfected cells (Fig. 4 A-E).

4. DISCUSSION

To date, 15 isoenzymes of the 17βHSD family have been described [5,6]. The extent to which individual enzymes of this family have been studied varies. The relevance of the 17βHSD14 protein in normal physiology has not been addressed, although we have previously shown tumoural expression of both *HSD17B14* mRNA and the 17βHSD14 protein to be of importance in breast cancer, both for predicting prognosis [21] and in predicting tamoxifen treatment response [22]. In both cases, 17βHSD14 appears to be a factor predicting good clinical outcome in breast cancer; however, functional mechanisms underlying this remain elusive. The current study was initiated in order to learn more of the normal physiology of the 17βHSD14 protein. An antibody targeting the 17βHSD14 antigen was rasied and used for immunohistochemical analysis of healthy human tissues. Furthermore, enzymatic properties of the protein in intact cells were assessed. Overall, distribution patterns and activity measurements of 17βHSD14 support the theory of the enzyme being an actor in intracrine sex steroid interconversion.

17βHSD14 was first cloned from a retinal cDNA library in 2000 by Haeseleer and Palczewski [1]. Based on amino acid sequence, the enzyme was predicted to utilize NADPH as a cofactor, which would render the enzyme reductive. However, using recombinant protein expressed in insect cells, the authors could not detect transfer of ^{3}H from [^{3}H]NADPH to any of the steroid substrates tested. Lukacik et al re-evaluated the same gene product in 2006 and also solved the crystal structure of 17βHSD14. The purified recombinant enzyme was screened against a compound library and was found to convert NAD+ to NADH in the presence of oestradiol, testosterone and 5-androstenediol. Oestradiol oxidation to oestrone was shown in a cell based system overexpressing *HSD17B14* [2]. In the current study, we utilized a similar cell based system and found significantly higher oxidation of both oestradiol and testosterone into oestrone and androstenedione respectively for transient *HSD17B14* overexpression compared with mock-transfected cells. The relevance of 17βHSD14 as a steroid-converting enzyme was strengthened by the immunohistochemical findings, which showed

presence of 17βHSD14 in several steroidogenic tissues including uterus, testis and breast. Among female and male reproductive tissues, 17βHSD14 was most strongly expressed in uterus and testis respectively, staining endometrial glands as well as male germ cells and Leydig cells, where 17βHSD14 may participate in the fine-tuning of the levels of bioactive sex hormones which is crucial for the homeostasis in these tissues. 17βHSD14 was not expressed in prostate tissue. Among a large number of normal breast samples analysed, 17βHSD14 was highly expressed in the majority. It has been suggested that the predominant enzymatic direction in normal breast is oxidative, favouring E2 to E1 conversion, whereas tumour progression is associated with a shift towards a reductive environment, and these changes have been shown to concur with changes in local protein expression of steroidogenic enzymes from a dominance of oxidative enzymes such as 17βHSD2, towards increased expression of reducing enzymes, e.g. 17βHSD1 [23-25].

17βHSD14 was expressed throughout the gastrointestinal tract with most prominent staining seen in stomach as well as in colonic and rectal samples. In colon and rectal samples, immunoreactivity of enterocytes and goblet cells of mucosal crypts was prominent in the outer absorptive border facing the lumen, whereas positivity towards the deeper situated glands gradually diminished. The same pattern, although not as marked, was seen in duodenal samples. Based on expression patterns in mucosal layers of the colon and rectum, which resemble those published regarding other oxidizing 17βHSD enzymes, e.g. type 2 and 4 in colon [26,27], it is suggestive that 17βHSD14 has a role in protecting tissues from exposure to excess reduced steroidal agents such as ingested or bacterially derived oestradiol. As proposed by English et al, the primary enzymatic activity in colonic tissue is oxidative, favouring the inactivation of potent sex steroids [27].

The kidney harbours several steroidogenic enzymes [28-30]. The exact significance of steroid metabolism in renal cells has not been extensively studied, although observed gender differences in e.g. blood pressure response to salt stimuli and the tendency to form urinary stones suggests involvement of sex steroids in the regulation of tubular reabsorption [28]. In our study, 17βHSD14 positivity in kidney was intense and specific, present in epithelial cells of proximal and distal tubules whereas renal corpuscles were negative. Specific immunopositivity for 17βHSD14 and other 17βHSD

1 enzymes such as 17βHSD5 in sites for selective ion and water transport in the kidney could possibly
2 suggest a role for 17βHSD enzymes in normal reabsorption physiology [28,31].

3 The *HSD17B14* gene product was first cloned from a retinal cDNA-library and was also found to be
4 highly expressed therein [1]. We found 17βHSD14 immunoreactivity in cytoplasmic projections of the
5 plexiform layers of the retina, whereas nuclear layers were negative, with an exception for the inner
6 nuclear layer, where staining was prominent in specific nuclei, most likely belonging to amacrine
7 cells. Interestingly, the inner nuclear layer of the retinal epithelium has been shown to harbour the
8 highest density of steroidogenic enzymes in rat retina [32]. The presence of 17βHSD14 in retina could
9 be related to a role in steroid metabolism, but it could also be related to a possible role in retinoid
10 metabolism. 17βHSD enzymes share homology with retinol dehydrogenase enzymes, and some
11 17βHSD enzymes have been shown to metabolise retinoids [16,33,34]. As recently shown by Haller *et*
12 *al*., the exchange of a single amino acid in the 17βHSD1 enzyme makes the enzyme efficiently
13 reducing all-*trans*-retinal to its alcohol [35].

14 Although conversion data support a role for 17βHSD14 in sex steroid metabolism, the conversion
15 rates of these reactions are relatively low, with approximately 8-15 % of the added hydroxysteroids
16 being oxidized after 72 hours. In comparison, and as a methodological control, using the same
17 transfection system, we transiently over-expressed *HSD17B2* and found 100% of added oestradiol to
18 be oxidised to oestrone within less than 24 hours. The low efficiency of 17βHSD14 in oxidising
19 hydroxysteroids could possibly suggest that the enzyme in addition operates on other, yet unknown
20 substrates. A role for 17βHSD14 in inflammatory responses is proposed by a recent interesting
21 publication by Saijo et al., in which 17βHSD14 is shown to act immunomodulatory by reducing
22 DHEA into androstenediol, which in turn is shown to be a significant inducer of anti-inflammatory
23 responses mediated by ERβ in the central nervous system [36]. When adding DHEA as a substrate to
24 transiently *HSD17B14* over-expressing MCF10A cells, we detected no reduction exceeding that of
25 mock-transfected cells within 72 hours using our HPLC-based system**.** The reason for this apparent
26 discrepancy remains elusive, and the suggestion that 17βHSD14 would potently act as a reductive
27 enzyme as shown by Saijo et al., is intriguing as neither we, nor Lukacik et al., could detect reductive

activity when adding ketosteroids such as E1 or A as substrates to purified enzyme [2] or cells over-expressing *HSD17B14*. It has been shown that oxidation or reduction reactions catalysed by 17βHSD enzymes are essentially reversible reactions, however, in intact cells these enzymes cause equilibrium, favouring either the 17β-hydroxy conformation or the 17-keto conformation of the sex steroid, and the enzymes are thus classified as either reductive or oxidative [37]. Sherbet et al., have shown that whereas the E2/E1 ratio in cell culture media of cells expressing *HSD17B1* could be altered by glucose deprivation which causes changes in the NADPH/NADP+ ratio and thus alters the preference from E1 to E2 as a substrate, the preference of *HSD17B2* for E2 oxidation was not affected by changes in the NAD+/NADH gradient [3]. The influence on the enzymatic properties of 17βHSD14 of factors that could affect cofactor availability, such as prolonged incubation times with subsequent changes in cell density and pH, have not been investigated, hence there remains a possibility that differences in cell culture conditions could be the cause of the dissimilar results. The discrepancy could also stem from the fact that a different cell line is used or even the means of analysis used which differs between our studies. Whereas Saijo et al used non-labelled steroids which after extraction from cell culture media were derivatised and then separated/analysed with gas chromatography, we used radiolabelled steroids which were separated via reversed phase HPLC and detected by an on-line scintillographer. Further studies on the topic of 17βHSD14 substrate preference are warranted.

In conclusion, the 17βHSD14 enzyme is expressed in several classical steroidogenic tissues such as breast, ovary and testis which supports the theory of 17βHSD14 being an actor in intracrine sex steroid interconversion. This theory is further strengthened by the finding that 17βHSD14 oxidises both oestradiol and testosterone in intact cells. Immunohistochemical staining patterns of 17βHSD14 with the enzyme being primarily expressed in glandular epithelial cells, may implicate a role for the enzyme in modulating the levels of secreted sex steroids, either endogenous or externally derived.

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LEGENDS

Figure 1

Immunoblot analysis of lysates from HEK293 (A) and MCF10A (B) cells over-expressing HSD17B14. A single band at 28 kDa corresponding to 17βHSD14 is seen in lane 2 of both blots. The band is not present in lanes 3 and 4 (row 2) representing mock-transfected and untransfected cells respectively. β-actin (upper row), at 45 kDa is used to control for equal loading.

Figure 2

17βHSD14 immunolocalisation in reproductive tissue; A, uterus, B, ovary, C, uterus, D, breast, E, testis and F, prostate. Scale bars represent 50 (C) or 100 (A, B, D, E, F) µm.

Figure 3

Immunolocalisation of 17βHSD14 in A, kidney, B, retina (arrows depicting inner nuclear layer), C stomach, D colon, E rectum and F duodenum. Peptide neutralisation assay; in G, liver section with17βHSD14 immunoreactivity and H, liver immunostained with an anti-17βHSD14 antibody neutralised with a 17βHSD14-peptide. Scale bars represent 50 (A, B, G, H) or 100 (C, D, E, F) µm.

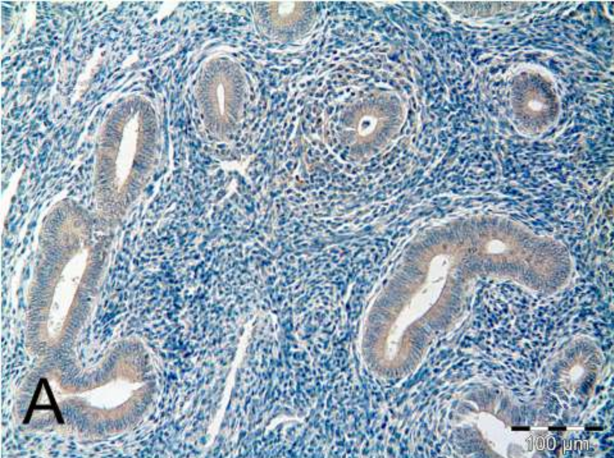
Figure 4

Interconversion of tritiated steroids in HEK293 (A to D) and MCF10A (E) transiently transfected with HSD17B14 expressed as the fraction of added compound compared to the total amount of added and generated steroid metabolite. Interconversion profiles for HSD17B14-transfected cells compared with that of a mock-transfected cells treated with (A) oestrone, (B) oestradiol, (C) androstenedione, (D) testosterone and (E) dehydroepiandrosterone (DHEA). Representative experiments are shown. Experiments have been performed in duplicates and repeated at least three times.

Table 1

Summary of immunohistochemical staining patterns and grading of 17βHSD14 in human tissue

2
3 1
50
β-actin
40
30
17βHSD14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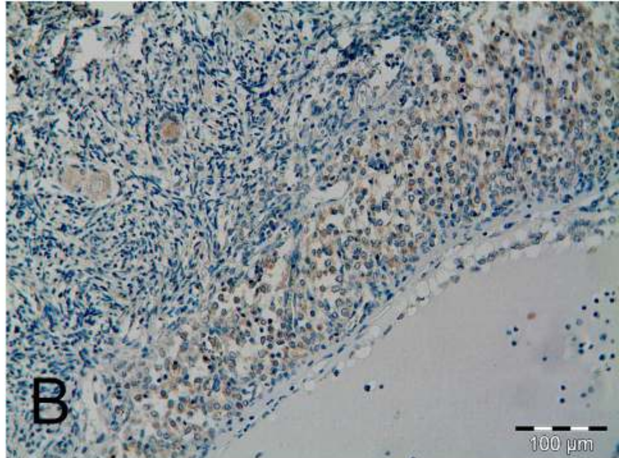
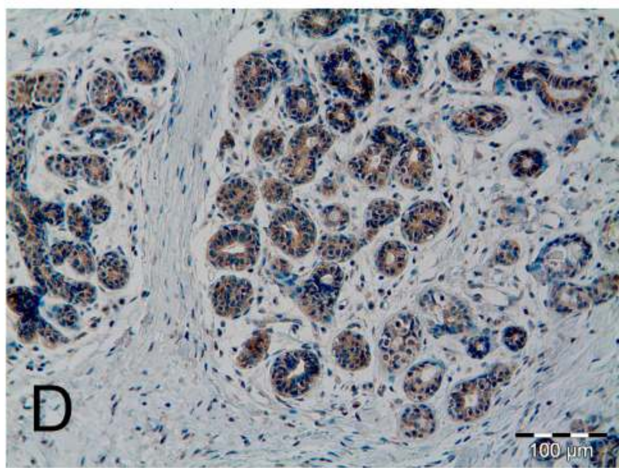

B
100 µm

C
50 µm

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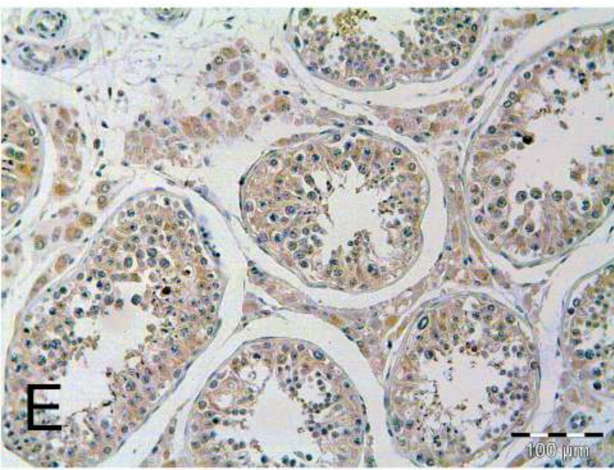
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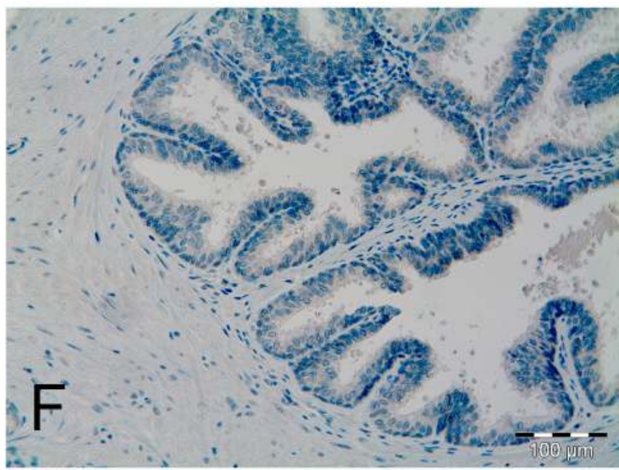
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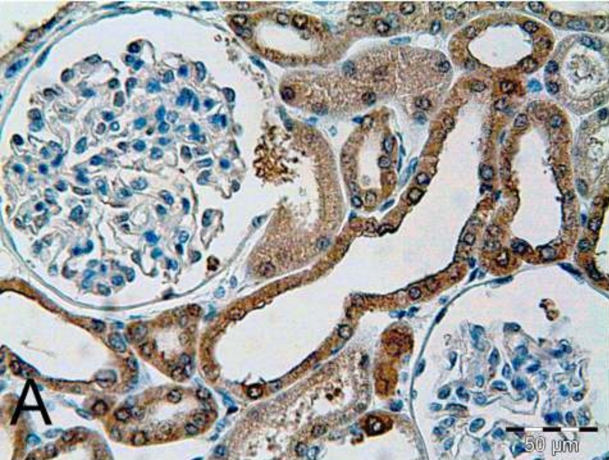
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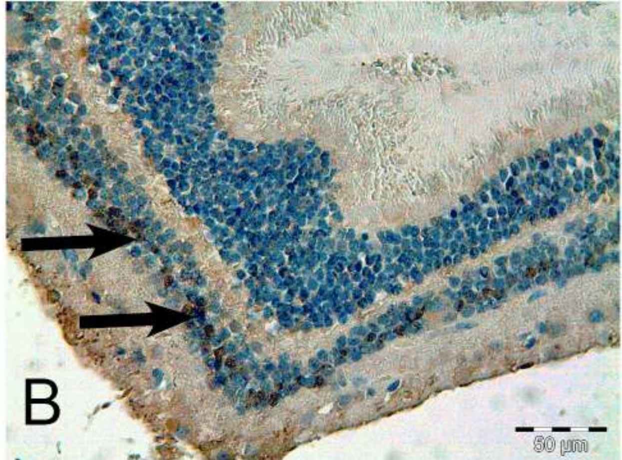
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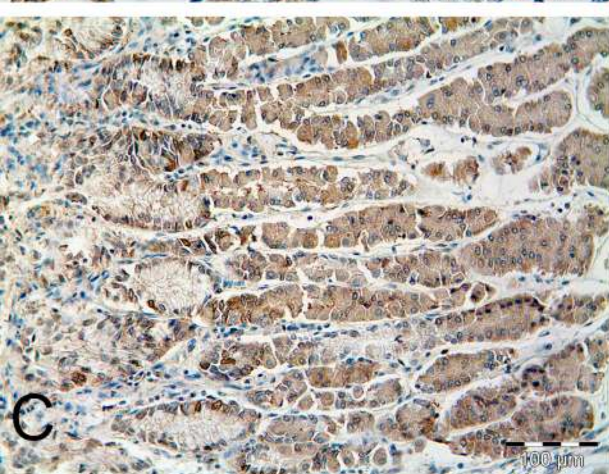
C
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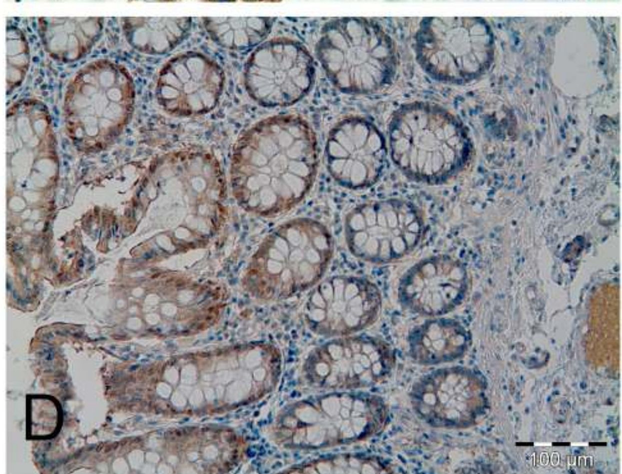
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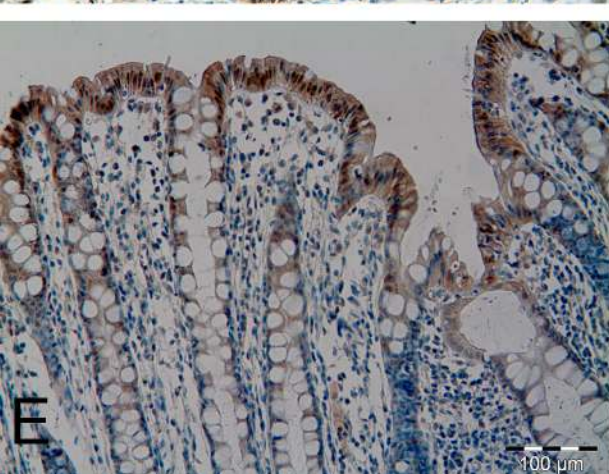
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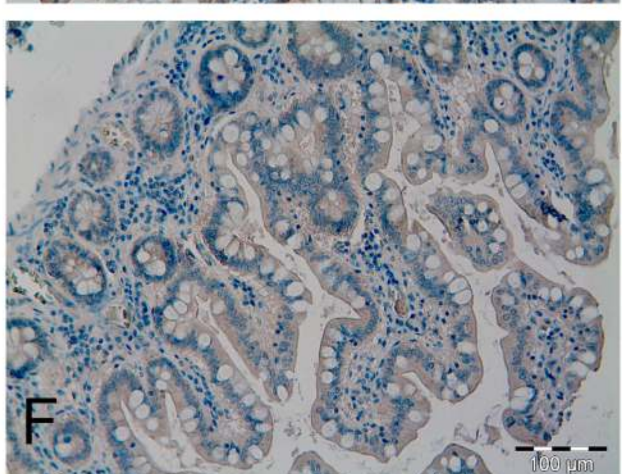
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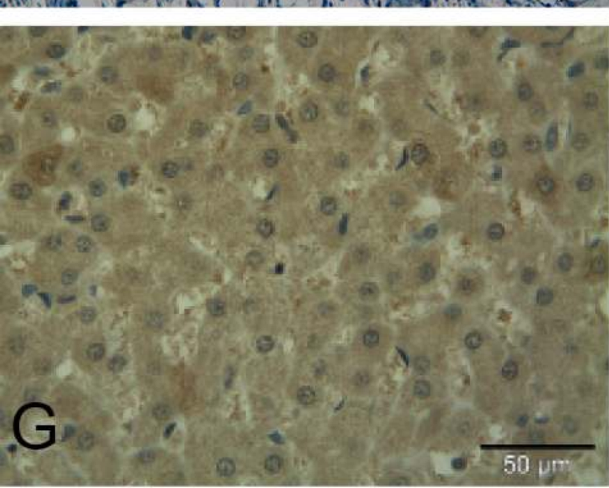
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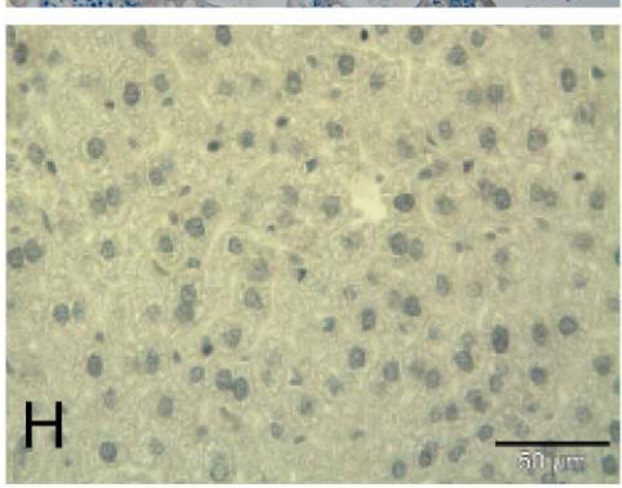
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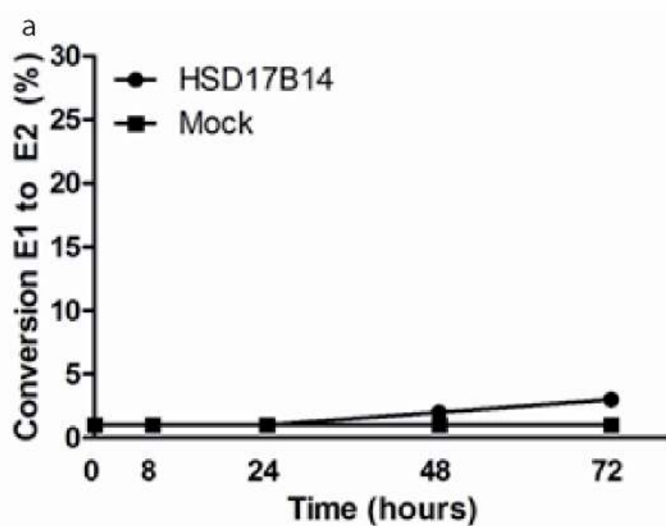
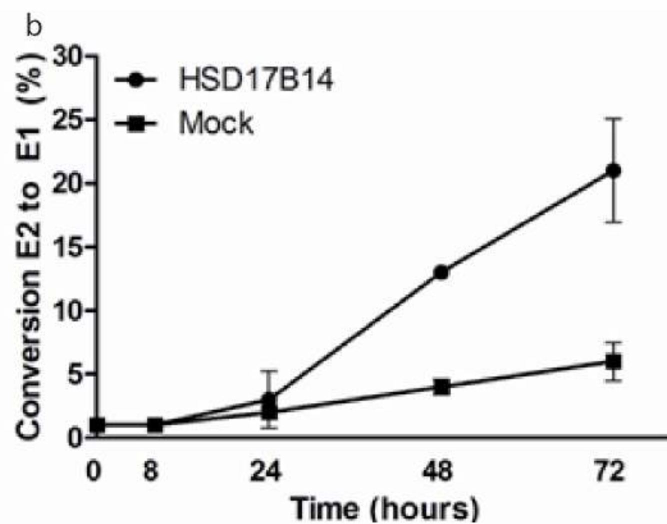
50 μm


a
Conversion E1 to E2 (%)
HSD17B14
Mock
Time (hours)
0
8
24
48
72
0
5
10
15
20
25
30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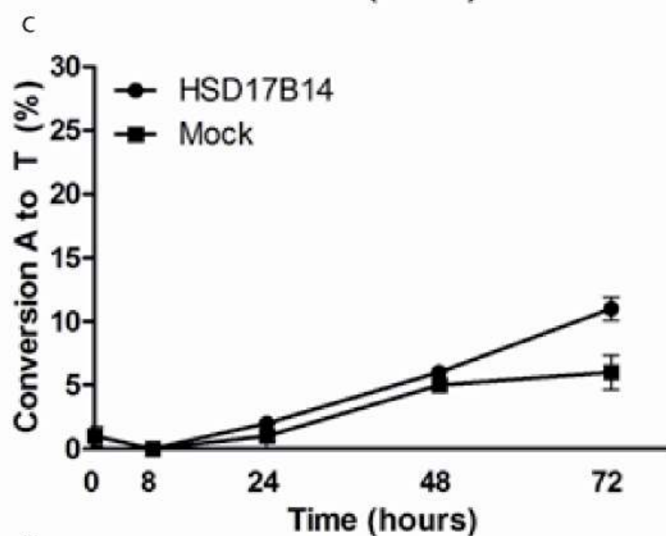
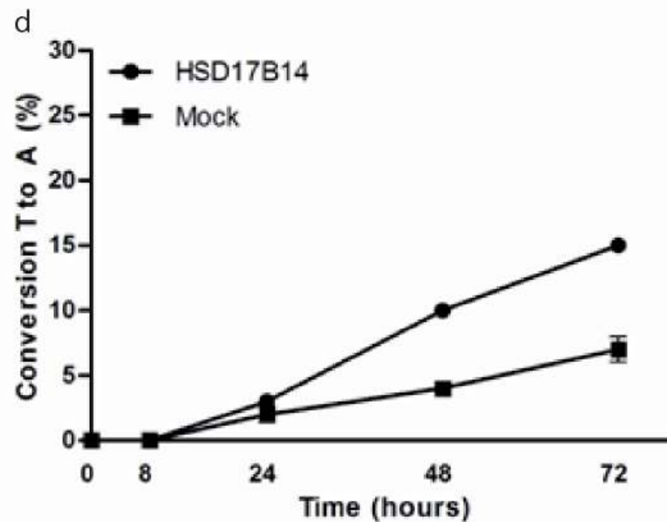
Conversion E2 to E1 (%)
HSD17B14
Mock
Time (hours)
0
8
24
48
72
0
5
10
15
20
25
30


c
Conversion A to T (%)
HSD17B14
Mock
Time (hours)
0
8
24
48
72
0
5
10
15
20
25
30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Conversion T to A (%)
HSD17B14
Mock
Time (hours)
0
8
24
48
72
0
5
10
15
20
25
30

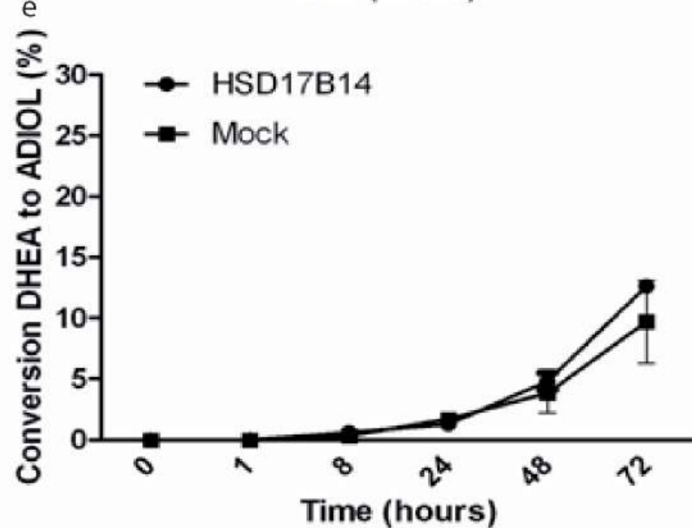
e
Conversion DHEA to ADIOL (%)
HSD17B14
Mock
Time (hours)
0
1
8
24
48
72
0
5
10
15
20
25
30

Table 1

Tissue	***Female n (median age)***	***Male n (median age)***	***Grading***	***Immunolocalisation***
Adrenal gland	2 (17.5)	1 (0,5)	Strong	*Cortex layer*
Bone (myeloid)	5 (50)	3 (56)	moderate	
Breast	60 (43.5)		Moderate/strong	*Ductal epithelial cells*
Brain	14 (24)		Moderate	*Gray and white matter*
Colon	19 (54)	41 (52)	Moderate/strong	*Glandular surface epithelium*
Eye	3 (52		Strong	*Retinal plexiform layers, inner nuclear layer amacrine cells*
Esophagus	1 (42)	7 (30)	Strong	*Mucosal cells*
Heart	3 (21)		Strong	
Kidney	5 (21)	3 (22)	Strong	*Cortical tubular epithelium*
Larynx		3 (45)	Moderate	*Outer mucosal layer*
Liver	6 (21)	2 (35)	Strong	*Glandular parenchyma*
Lung	2 (21)	6 (39.5)	Weak	*Epithelial cells in bronchial areas*
Lymph node	2 (38.5)	3 (61)	Negative	
Mesothelium	2 (21)	1 (43)	Weak	
Nerve	2 (18)	1 (43)	Weak	
Ovary	8 (21)		Weak	*Dominant follicles*
Pancreas	6 (21)	2(20.5)	Moderate/strong	*Acinar cells*
Parathyroid gland		3 (33)	Moderate/strong	
Pituitary	2 (16.5)	1 (56)	Moderate/strong	
Prostate		60 (66)	Weak	*Tubuloalveolar epithelium*
Rectum	1 (35)	4 (50)	Strong	*Gland cell surface epithelium*
Salivary Gland	1 (38)	2 (36)	Strong	
Skeletal muscle		3 (40)	Strong	
Skin	1 (50)	7 (35)	Moderate	*Stratum spinosum and glandular structures*
Small Intestine	2 (49)	3 (40)	Moderate	*Mucosal cells*
Spleen	4 (21)	4 (32.5)	Moderate	
Stomach	3 (14)	5 (35)	Moderate/strong	*Glandular*
Testis		8 (40)	Strong	*Spermatogonia, spermatocytes, Leydig cells*
Thymus gland	2 (18)	6 (2)	Moderate	*Centers of Hassals' corpuscles*
Thyroid	1 (21)	2 (37.5)	Weak	
Tonsil	2 (21)	1 (48)	Negative	
Uterus	3 (21)		Moderate/strong	*Epithelial cells of endometrial glands*