**IHC Protocol for New Antibody Requests**

Requesting Pathologist: William McDonald, MD Date: 8/28/18

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| To be completed by the Requesting Pathologist: | |
| Name of Antibody: (including clone) | T-Pit  Atlas Antibodies Product Name Anti-TBX19  Product Number AMAb91409  Clone Number CL6251 |
| Intended use: | **-Pit IMMUNOHISTOCHEMISTRY (IHC)**  **Background:**  Pituitary adenomas can be classified in three broad families based on their resemblance to developmental pathways of the anterior pituitary (1). While immunohistochemical stains for the six anterior pituitary hormones (prolactin, growth hormone, thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone and adrenocorticotropin (ACTH)) are widely used, some of these suffer from suboptimal sensitivity and specificity. ACTH immunoreactivity in particular is widely regarded as lacking in sensitivity and specificity. This is problematic, since ACTH-producing macroadenomas are considered more aggressive(2), and the resection of hormonally active ACTH-producing tumors can cause life-threatening cortisol insufficiency if left untreated. That is, both over- and under-detection of ACTH-producing tumors can have clinical consequences.  ACTH-producing adenomas account for approximately 17% of pituitary adenomas (3) . T-box transcription factor (T-Pit) mediates the corticotroph pathway (4, 5). Until recently, immunostains for T-Pit were restricted to frozen material, but the introduction of a polyclonal antibody to T-pit developed and validated according to standardized procedures within the Human Protein Atlas (<http://www.proteinatlas.org>) provides a reagent that reportedly overcomes this restriction.  **T-Pit Immunohistochemistry, Rationale and Clinical Significance:**  T-Pit IHC is used in conjunction with IHC for SF-1 and Pit-1 to classify pituitary adenomas. According to Sjostedt et al. (6), T-Pit IHC is more sensitive and specific than ACTH IHC.  **Use of T-Pit Immunohistochemistry:**  T-Pit IHC is useful in the setting of sellar mass. Two types of corticotroph adenoma are generally encountered: small, hormonally active adenomas that are associated with Cushing’s disease and larger, more aggressive, typically hormonally silent macroadenomas. The presence of T-Pit nuclear reactivity within a monomorphous population of neuroendocrine cells within the sella turcica strongly supports adrenocorticotropin lineage.  Normal adenohypophysis also shows immunoreactivity in scattered cells and clusters of cells, corresponding to the normal corticotropin-producing cells. In conjunction with SF-1 and Pit-1, T-Pit “rounds out” the family classification of pituitary adenomas.  **Specimen Requirements:**  Formalin-fixed, paraffin-embedded samples.  **Validation:**  Validation will be based largely upon materials examined during the validation of a standard protocol for the IHC characterization of pituitary adenomas at Allina Health Laboratories, as recently published (7). In that work, we showed that a screening panel comprised of SF-1, Pit-1 and ACTH, followed by IHC for PRL, GH, TSH, and CAM5.2 for Pit-1 family members or plurihormonal tumors provided more accurate diagnoses using approximately one-third fewer IHC stains (7). Since clinical and serological data were also collected for the original studies, we will be able to test all of our T-Pit IHC findings against the clinical and serological setting. |
| Will this Antibody replace another currently in use? | It will replace ACTH in routine use, although I anticipate maintaining ACTH for reference customers and troubleshooting. |
| Anticipated annual volume:  (# cases per year) | 30-50 cases per year |
| References from literature showing use of antibody which include testing information (Testing platform used, dilution used, etc.): | **References:**  1. Asa SL. Practical pituitary pathology: what does the pathologist need to know? Arch Pathol Lab Med. 2008;132(8):1231-40.  2. Asa S. Tumors of the Pituitary Gland. Silverberg SG, editor. Washington, DC: American Registry of Pathology; 2011. 283 p.  3. Mete O, Cintosun A, Pressman I, Asa SL. Epidemiology and biomarker profile of pituitary adenohypophysial tumors. Mod Pathol. 2018;12(10):018-0016.  4. Lamolet B, Pulichino AM, Lamonerie T, Gauthier Y, Brue T, Enjalbert A, et al. A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. Cell. 2001;104(6):849-59.  5. Pulichino AM, Vallette-Kasic S, Couture C, Gauthier Y, Brue T, David M, et al. Human and mouse TPIT gene mutations cause early onset pituitary ACTH deficiency. Genes Dev. 2003;17(6):711-6.  6. Sjostedt E, Bollerslev J, Mulder J, Lindskog C, Ponten F, Casar-Borota O. A specific antibody to detect transcription factor T-Pit: a reliable marker of corticotroph cell differentiation and a tool to improve the classification of pituitary neuroendocrine tumours. Acta Neuropathol. 2017;134(4):675-7. doi: 10.1007/s00401-017-1768-9. Epub 2017 Aug 19.  7. McDonald WC, Banerji N, McDonald KN, Ho B, Macias V, Kajdacsy-Balla A. Steroidogenic Factor 1, Pit-1, and Adrenocorticotropic Hormone: A Rational Starting Place for the Immunohistochemical Characterization of Pituitary Adenoma. Arch Pathol Lab Med. 2017;141(1):104-12.  See also University of Virginia Pathology Protocol for T-PIT: |
| Outside pathologist contacts with experience using antibody  (if applicable): | Bea Lopes, MD/PhD  https://uvahealth.com/findadoctor/profile/maria-beatriz-lopes |

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| To be completed by the IHC Lab: | |
| Antibody Vendor: |  |
| Antibody Type: (monoclonal, polyclonal |  |
| Antibody Source: (mouse, rabbit, goat) |  |
| Antibody Cost & Vial Volume: |  |
| Additional costs: (kits, amplifiers, etc.) |  |
| Suggested Platform based on  Literature Review: |  |
| Suggested Antigen Retrieval based on Literature Review: |  |
| Suggested Dilution based on  Literature Review: |  |

Request Approved or Denied:

Reason(s) for Denial: