# **Product Datasheet**

# Aromatase Antibody - BSA Free NB100-1596

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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## NB100-1596

Aromatase Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	55 kDa
Product Description	
Host	Rabbit
Gene ID	1588
Gene Symbol	CYP19A1
Species	Human, Mouse, Rat, Primate, Rabbit
Reactivity Notes	Use in Rabbit reported in scientific literature (PMID:35245530). Rabbit reactivity reported in scientific literature (PMID: 25850953). Immunogen displays the following percentage of sequence identity for non-tested species: horse (91%), cow (90%), pig (83%), sheep (81%), goat (81%), and dog (81%) proteins. Rat reactivity reported in scientific literature (PMID: 29898754). Use in Mouse reported in scientific literature (PMID:32634520).
Immunogen	A synthetic peptide made to a C-terminal portion of the human Aromatase protein (between residues 400-502). [UniProt# P11511]
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1 ug/ml, Simple Western reported in scientific literature (PMID 29898754), Immunohistochemistry 1:200. Use reported in scientific literature (PMID 28133606), Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:200
Application Notes	In Western blot, a band is seen at ~55 kDa in human brain, and Immunocytochemistry/Immunofluorescence where membrane staining is observed in SH-SY-5Y cells. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

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Immunohistochemistry-Paraffin: Aromatase Antibody [NB100-1596] -Expression of the cytochrome P450 aromatase in the ovary of hypothyroid (Hypo; solid bar; n = 6) rabbits. Immunoreactivity of antiaromatase (+) was identified in granulosa cells of antral follicles (tertiary, tf; Graafian, gf) of hypothyroid ovaries. Nonlabeling was observed when the primary antibody was omitted. Bars: 100 um (1st image); and 20 um (2nd image). Granulose cells, gc; theca cells, tc. Image collected and cropped by CiteAb from the following publication (https://www.hindawi.com/journals/bmri/2017/3795950/), licensed under a CC-BY license.

Simple Western: Aromatase Antibody [NB100-1596] - Aromatase expression by Western blotting (WES system) from in vitro activated M1 and M2 rat macrophages using Aromatase antibody. Controls include ovary (positive control) and ovary in which the primary antibody was preincubated with an aromatase blocking peptide for 1 h, as well as KGN cells with (positive control) and without (negative control) forskolin treatment. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/29898754/) licensed under a CC-BY license.

Immunohistochemistry: Aromatase Antibody [NB100-1596] - Expression of the cytochrome P450 aromatase in the ovary of control (C; open bar; n = 6) & hypothyroid (Hypo; solid bar; n = 6) rabbits. Representative immunoblot showing the expression of aromatase (a) & Ponceau's Red stained membrane (b). Relative expression of aromatase in C & Hypo groups (c). Data are mean  $\pm$  SEM.  $\Box \Box P < 0.01$ . Immunoreactivity of antiaromatase (+) was identified in granulosa cells of antral follicles (tertiary, tf; Graafian, gf) of control (d, e) & hypothyroid (f, g) ovaries. Nonlabeling was observed when the primary antibody was omitted (negative control; h). Bars: (d) 200 µm; (f) & (h) 100 µm; & (e) & (g) 20 µm. Granulose cells, gc; theca cells, tc. Image collected & cropped by CiteAb from the following publication

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(d)



(e)



(e)



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Simple Western: Aromatase Antibody [NB100-1596] - Metformin decreases aromatase-positive, tumor-associated macrophages. a Quantification of aromatase-positive cells & b CD68-positive (CD68+) macrophages costaining for aromatase in the tumor border of tissues from control or metformin-treated rats. c Representative control & metformin-treated IHC images of CD68 & aromatase dual staining in tissue bordering mammary tumors (T) (arrows: yellow = CD68+aromatase+, brown = CD68+; scale bar = 50um). d Aromatase expression by Western blotting (WES system) from in vitro activated M1 & M2 rat macrophages using two different primary antibodies (Ab #1: Novus NB100-1596; Ab #2: clone 677, Baylor College of Medicine). Controls include ovary (positive control) & ovary in which the primary antibody was preincubated with an aromatase blocking peptide for 1 h, as well as KGN cells with (positive control) & without (negative control) forskolin treatment. e IHC intensity score for ER & PR (0 = no stain; 1 = weak; 2 = moderate; 3 = strong staining). \*p < 0.05 Image collected & cropped by CiteAb from the following publication (https://breast-cancerresearch.biomedcentral.com/articles/10.1186/s13058-018-0974-2), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





#### **Publications**

Uddin MM, Ibrahim MMH, Briski KP Sex-dimorphic neuroestradiol regulation of ventromedial hypothalamic nucleus glucoregulatory transmitter and glycogen metabolism enzyme protein expression in the rat BMC Neurosci 2020-11-25 [PMID: 33238883]

Ibrahim MMH, Bheemanapally K, Sylvester PW, Briski KP. Norepinephrine Regulation of Adrenergic Receptor Expression, 5' AMP-Activated Protein Kinase Activity, and Glycogen Metabolism and Mass in Male Versus Female Hypothalamic Primary Astrocyte Cultures ASN Neuro 2020-11-12 [PMID: 33176438] (In vivo assay)

Briski KP, Napit PR, Alhamyani A et al. Sex-Dimorphic Octadecaneuropeptide (ODN) Regulation of Ventromedial Hypothalamic Nucleus Glucoregulatory Neuron Function and Counterregulatory Hormone Secretion ASN neuro 2023 -05-17 [PMID: 37194319] (WB, Rat)

Briski KP, Mahmood ASMH, Uddin MM et al. Effects of Ventromedial Hypothalamic Nucleus (VMN) Aromatase Gene Knockdown on VMN Glycogen Metabolism and Glucoregulatory Neurotransmission Biology 2023-02-03 [PMID: 36829519] (Western Blot, Rat)

Wang H, Feng X, Wang T et al. Role and mechanism of the p-JAK2/p-STAT3 signaling pathway in follicular development in PCOS rats General and comparative endocrinology 2022-10-03 [PMID: 36202220] (WB, Rat)

Kata D, GrOf I, Hoyk Z et al. Immunofluorescent Evidence for Nuclear Localization of Aromatase in Astrocytes in the Rat Central Nervous System International journal of molecular sciences 2022-08-11 [PMID: 36012212] (IHC-P, IHC-F, ICC/IF, Rat, Human)

Details:

Dilution used 1:100

Rodriguez-Castelan J, Zepeda-Perez D, Rojas-Juarez R et al. Effects of hypothyroidism on the female pancreas involve the regulation of estrogen receptors Steroids 2022-03-01 [PMID: 35245530] (WB, Rabbit)

Abbas M, Alqaisi K, Disi A, Hameed N Chrysin increased progesterone and LH levels, estrous phase duration and altered uterine histology without affecting aromatase expression in rat ovary Journal of Functional Foods 2022-02-01 (IHC-P, Rat)

Ganesan S, Keating AF Ovarian mitochondrial and oxidative stress proteins are altered by glyphosate exposure in mice Toxicol. Appl. Pharmacol. 2020-07-04 [PMID: 32634520] (WB, Mouse)

Ganesan S, McGuire BC, Keating AF Absence of glyphosate-induced effects on ovarian folliculogenesis and steroidogenesis Reprod. Toxicol. 2020-06-24 [PMID: 32592754] (ICC/IF, WB, Mouse)

Giles ED, Jindal S, Wellberg EA et al. Metformin inhibits stromal aromatase expression and tumor progression in a rodent model of postmenopausal breast cancer Breast Cancer Res. 2018-06-14 [PMID: 29898754] (Simple Western, Rat)

Rodriguez-Castelan J, Mendez-Tepepa M, Carrillo-Portillo Y et al. Hypothyroidism Reduces the Size of Ovarian Follicles and Promotes Hypertrophy of Periovarian Fat with Infiltration of Macrophages in Adult Rabbits Biomed Res Int 2017-01-30 [PMID: 28133606] (IF/IHC, WB, Rabbit)

More publications at <a href="http://www.novusbio.com/NB100-1596">http://www.novusbio.com/NB100-1596</a>



#### **Procedures**

Western Blot protocol for Aromatase Antibody (NB100-1596) Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.

2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.

3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.

4. Rinse the blot TBS -0.05% Tween 20 (TBST).

5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.

6. Wash the membrane in TBST three times for 10 minutes each.

7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.

8. Wash the membrane in TBST three times for 10 minutes each.

9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.

10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).

11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/Immunofluorescence protocol for Aromatase Antibody (NB100-1596) Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.

2. Remove the formalin and wash the cells in PBS.

3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.

4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.

5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.

6. Add primary antibody at appropriate dilution and incubate overnight at 4C.

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7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.

8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.

9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.

10. Counter stain DNA with DAPi if required.



#### Immunohistochemistry-Paraffin Protocol for Aromatase Antibody (NB100-1596)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

- 1. Wash sections in deionized water three times for 5 minutes each.
- 2. Wash sections in PBS for 5 minutes.
- 3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
- 4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
- 5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
- 6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
- 7. Wash sections three times in wash buffer for 5 minutes each.
- 8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
- 9. As soon as the sections develop, immerse slides in deionized water.
- 10. Counterstain sections in hematoxylin.
- 11. Wash sections in deionized water two times for 5 minutes each.
- 12. Dehydrate sections.
- 13. Mount coverslips.





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# Products Related to NB100-1596

NBL1-09674	Aromatase Overexpression Lysate
NB100-1596PEP	Aromatase Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

### Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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