

# Product Datasheet

## DUX4 Antibody (P4H2) - BSA Free NBP1-49552

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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**NBP1-49552**

DUX4 Antibody (P4H2) - BSA Free

| Product Information         |                                                                                                                                                                                                                                                                                                                                                                                     |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Unit Size                   | 0.1 ml                                                                                                                                                                                                                                                                                                                                                                              |
| Concentration               | 1 mg/ml                                                                                                                                                                                                                                                                                                                                                                             |
| Storage                     | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.                                                                                                                                                                                                                                                                                              |
| Clonality                   | Monoclonal                                                                                                                                                                                                                                                                                                                                                                          |
| Clone                       | P4H2                                                                                                                                                                                                                                                                                                                                                                                |
| Preservative                | 0.02% Sodium Azide                                                                                                                                                                                                                                                                                                                                                                  |
| Isotype                     | IgG1                                                                                                                                                                                                                                                                                                                                                                                |
| Purity                      | Protein G purified                                                                                                                                                                                                                                                                                                                                                                  |
| Buffer                      | PBS                                                                                                                                                                                                                                                                                                                                                                                 |
| Target Molecular Weight     | 52 kDa                                                                                                                                                                                                                                                                                                                                                                              |
| Product Description         |                                                                                                                                                                                                                                                                                                                                                                                     |
| Host                        | Mouse                                                                                                                                                                                                                                                                                                                                                                               |
| Gene ID                     | 100288687                                                                                                                                                                                                                                                                                                                                                                           |
| Gene Symbol                 | DUX4                                                                                                                                                                                                                                                                                                                                                                                |
| Species                     | Human                                                                                                                                                                                                                                                                                                                                                                               |
| Specificity/Sensitivity     | This antibody is specific for DUX4 and does not recognize DUX4c.                                                                                                                                                                                                                                                                                                                    |
| Immunogen                   | Synthetic peptide corresponding to the C-terminus of human DUX4. [Swiss-Prot: Q9UBX2]                                                                                                                                                                                                                                                                                               |
| Product Application Details |                                                                                                                                                                                                                                                                                                                                                                                     |
| Applications                | Western Blot, Simple Western, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin                                                                                                                                                                                                                      |
| Recommended Dilutions       | Western Blot 0.5-2.0ug/ml, Simple Western 1:10, ELISA reported in scientific literature (PMID 21529284), Immunohistochemistry 1:100-1:200, Immunocytochemistry/ Immunofluorescence 1:100-1:200, Immunohistochemistry-Paraffin 1:100-1:200, Immunohistochemistry-Frozen 1:100-1:200                                                                                                  |
| Application Notes           | In Western blot, a band can be seen at approx. 52 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:10, apparent MW was 61 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. |

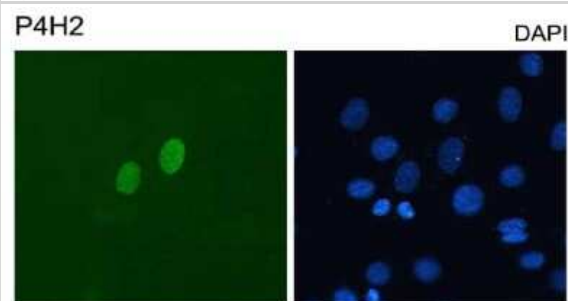


## Images

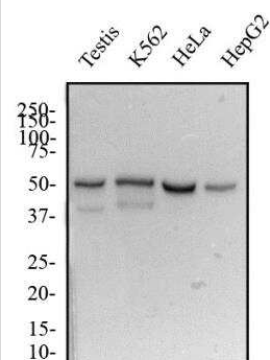
**Simple Western: DUX4 Antibody (P4H2) [NBP1-49552]** - Lane view shows a specific band for DUX4 in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



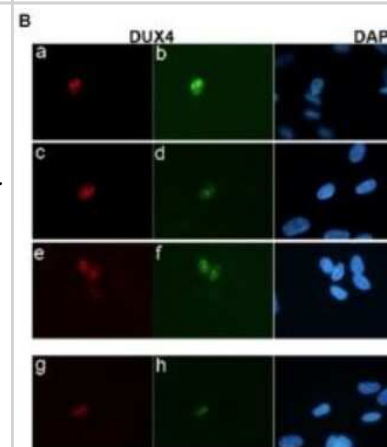
**Immunocytochemistry/Immunofluorescence: DUX4 Antibody (P4H2) [NBP1-49552]** - Detection of human DUX4 on C2C12 mouse myoblasts transfected with pCS2-DUX4. Cells were counterstained with DAPI for nuclei.



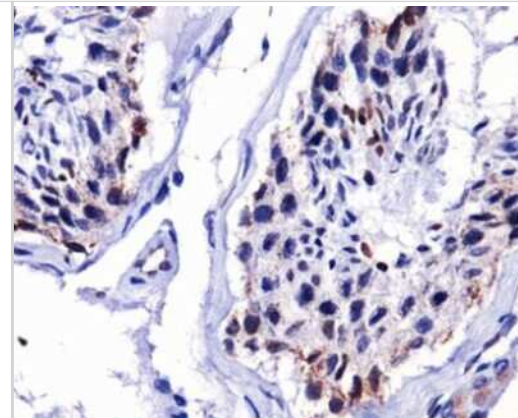
**Western Blot: DUX4 Antibody (P4H2) [NBP1-49552]** - Total protein from human testis tissue and human K562, HeLa and HepG2 cell lines was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-DUX4 in 1% non-fat milk in TBST and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



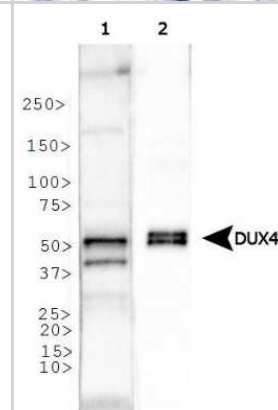
**Immunocytochemistry/Immunofluorescence: DUX4 Antibody (P4H2) [NBP1-49552]** - A small number of FSHD muscle cells express a relatively large amount of DUX4. Cultured FSHD muscle cells were differentiated and immunostained with mAbs to DUX4. Cells were co-stained with the E5-5 rabbit mAb to the DUX4 C-terminal region (panels a, c, e) and the P2G4 mouse mAb to the N-term. region (panels b,d,f), or co-stained with the P4H2 mouse mAb to the C-terminal region and the E14-3 rabbit mAb to the N-term. region. Approximately 1 cell per 1000 showed nuclear staining and the co-localization of both the N-term. and C-term. regions indicates that these cells are expressing the full-length DUX4 protein. No positive nuclei were apparent in the control muscle cultures. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pgen.1001181>), licensed under a CC-BY license.



Immunohistochemistry: DUX4 Antibody (P4H2) [NBP1-49552] - Analysis of DUX4 on human testis



Western Blot: DUX4 Antibody (P4H2) [NBP1-49552] - Analysis of DUX4 expression in (1) human testis and (2) DUX4 protein lysates.



## Publications

Ganassi M, Figeac N, Reynaud M et al. Antagonism Between DUX4 and DUX4c Highlights a Pathomechanism Operating Through  $\beta$ -Catenin in Facioscapulohumeral Muscular Dystrophy *Frontiers in Cell and Developmental Biology* 2022-09-07 [PMID: 36158201] (Western Blot, Block/Neutralize)

Rashnonejad A, Chermahini G, Taylor N et al. Designed U7 small nuclear RNA antisense expression cassettes inhibit DUX4 expression and improve FSHD-associated outcomes in DUX4 over-expressing cells and FSHD patient myotubes *Molecular Therapy - Nucleic Acids* 2020-12-01 [PMID: 33510937] (WB, Human)

Choi IY, Lim H, Estrellas K, et al. Concordant but Varied Phenotypes among Duchenne Muscular Dystrophy Patient-Specific Myoblasts Derived using a Human iPSC-Based Model. *Cell Rep.* 2016-06-07 [PMID: 27239027]

Narayanaswamy PB, Baral TK, Haller H et al. Transcriptomic pathway analysis of urokinase receptor silenced breast cancer cells: a microarray study. *Oncotarget* 2017-11-24 [PMID: 29254187] (WB, Human)

Geng LN, Tyler AE, Tapscott SJ. Immunodetection of human double homeobox 4. *Hybridoma (Larchmt)*;30(2):125-30. 2011-04-01 [PMID: 21529284] (ICC/IF, ELISA, WB, Human)

Snider L, Geng LN, Lemmers RJ et al. Facioscapulohumeral dystrophy: incomplete suppression of a retrotransposed gene. *PLoS Genet* 2010-10-01 [PMID: 21060811] (ICC/IF, WB, Human)

## Procedures

### Western Blot protocol Specific for DUX4 Antibody (P4H2)

DUX4 Antibody (P4H2):

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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 manufacturers instructions.

\*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

### Immunocytochemistry/Immunofluorescence Protocol for DUX4 Antibody (NBP1-49552)

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 permeabilization 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.
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 DUX4 Antibody (NBP1-49552)****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 all the time).

**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 NBP1-49552**

|                  |                                                         |
|------------------|---------------------------------------------------------|
| HAF007           | Goat anti-Mouse IgG Secondary Antibody [HRP]            |
| NB720-B          | Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] |
| NBP1-97005-0.5mg | Mouse IgG1 Isotype Control (MG1)                        |
| NBP1-49552B      | DUX4 Antibody (P4H2) [Biotin]                           |

### **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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