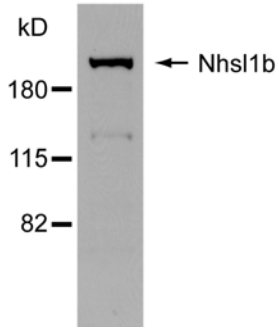
Product Data Sheet

Product Name:	**Anti-Nhsl-1a/1b (IN-2), Z-Fish™** *(NHS-like 1a, 1b)*
Catalog Number:	55634s
Lot Number:	See label on vial
Product Description:	This rabbit polyclonal antibody is supplied as an epitope-affinity purified rabbit IgG in 100 µl of 40 mM MOPS buffer (pH 7.5) containing 0.1% BSA 50% glycerol, and 0.05% NaN_3.
Immunogen:	A synthetic peptide derived from the intermediate region of zebrafish Nhsl-1a protein (GenBank accession # XP_695071.4).
Species Reactivity:	The species reactivity is exclusively to zebrafish. The antibody reactivity was validated by ELISA. The specificity was confirmed by Western blotting analysis in zebrafish tissue lysates.
Application Notes:	The following concentration ranges are recommended starting points for this product. The investigator should determine the optimal working concentrations for specific applications.

ELISA for immunizing peptide:	1: 5,000-20,000
Western blot:	1: 500-1,000



Nhsl-1b expressed in HEK293T cells blotted with the anti-Nhsl-1a/1b (IN-2) (cat#55634) antibody at 1:1000.

Storage:	Store at -20 °C for up to 24 months upon receiving the product.
Related Products:	Anti-Nhsl-1a/1b (IN-2) blocking peptide, Z-Fish™, Cat# 55634P

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Protocols:

Western blot

1. Run SDS-PAGE gel, and then Western transfer the protein samples to nitrocellulose (NC) or PVDF (need to pre-activate by soaking the membrane in 100% methanol for 10 minutes) membrane for immunoblot analysis. Use pre-stained molecular markers to indicate the size and transferring efficiency.

    - For those antibodies with a low expression level, load as much as possible of the samples on the SDS-PAGE gel.
    - Try to do the transfer for an extended period at low temperature (for example, 25V, overnight at 4 °C) to get the best transferring result.

2. Block the membrane with blocking buffer (made with 5% non-fat milk in 1x TBST) for 60 minutes at room temperature.
3. Dilute the primary antibody with blocking buffer according to the suggested dilution factor on datasheet.
4. Remove the blocking buffer and add enough diluted primary antibody to cover the membrane.
5. Incubate the membrane with primary antibody for 1hr at R/T with rocking. You can also do overnight incubation at 4 °C, but make sure you cover the western-blot tray to prevent excessive evaporation.

    - Overnight incubation at 4 °C is recommended to obtain better signal/background ratio.

6. Briefly wash the membrane with 1xTBST once to remove any excessive primary antibody.
7. Wash the membrane with 1xTBST 3 times for 5, 5, and 15 minutes.

    - If the background is high, wash with high salt TBST (0.5 M NaCl in 1x TBST) instead of regular 1xTBST.

8. In this antibody characterization, we used goat anti-rabbit IgG conjugated with Hilyte Fluor™ 750 (cat#61056-H750) with dilution 1:20,000. If other secondary antibody (for example, HRP-conjugated secondary antibody) is used, dilute with blocking buffer accordingly.
9. Incubate the membrane with secondary antibody for 60 minutes at R/T.
10. Wash the membrane with 1xTBST briefly, and then 4 times (5 min/5 min/15 min/15 min). To get better results in the high background cases, wash with high salt TBST (0.5 M NaCl in 1x TBST).
11. If HRP-conjugated secondary antibody is used, prepare the chemiluminescence development substrate mixture by mixing equal amount of solutions 1 and 2.
12. Image development.

Zebrafish Embryo Whole-Mount IHC

PROCEDURE

1. Fixation & storage of embryos

1.1 Removal of chorions for embyos older than 18 somites (older than 18 hpf).

a. Collect 200~300 (up to 500) staged zebrafish embryos into a 3.5 cm Petri dish, remove all the culture media.

b. Add 1 ml of pronase (2 mg/ml), gently shake and incubate at 37 °C for 15 ~ 20 min (or until all the embryo shells are broken). Frequently check the embryos during the incubation to monitor the break of the egg shells.

c. Remove all the pronase and the broken shells with a pipette, rinse the dechorionated embryos 3x5 min (three times at five minutes each) with E2 buffer.

1.2 Transfer all the embryos into a 5 ml capped glass vial. Put the vial on ice until all are unconscious and settled on the bottom of the vial. Remove the entire E2 buffer.

- For PFA fixation: re-suspend the embryos with ice-cold 4% PFA;
- For Dent's fixation: suspend the embryos with –20 °C Dent's fixative (80% Methanol, 20% DMSO);
- For TCA fixation: suspend the embryos with ice-cold 10% TCA.

Incubate overnight at 4°C.

1.3 Remove fixatives. Rinse the embryos with PBSTx 3x10 minutes. These embryos can be used immediately for antibody staining.

1.4 For embryo storage, remove the buffer and add 100% ice-cold methanol. Store at -20°C up to several months.

2. Antibody staining (Day 1)

2.1 Rehydration. Transfer determined amount of embryos into a 5 ml glass vial. Remove the methanol and rehydrate them by successive incubations in the following solutions (25°C, 1 ml/well):

75% MeOH/25% PBS for 5 min (no agitation)
50% MeOH/50% PBS for 5 min (no agitation)
25% MeOH/75% PBS for 5 min (no agitation)
100% PBSTx for 5 min (with rocking agitation)

2.2 Blocking. Re-suspend the embryos with 1 ml of blocking buffer 1 and incubate for 3-4 hours at room temperature (or 4 °C overnight) with rocking agitation.

2.3 Primary antibody staining. Transfer the desired number of embryos (5~6) into each well of a 24-well plate(s). Add 0.5 ml of blocking buffer 2 to each well. Add calculated amount of primary antibodies into the wells.

2.4 Incubate the plate(s) overnight at 4 °C with rocking agitation.

3. Antibody staining (Day 2)

3.1 Wash at room temperature with rocking agitation:

a. PBSTx, very brief wash
b. PBSTx for 3x5 min
c. PBSTx for 3x20 min

3.2 Add diluted (1:5000 in blocking buffer 2) secondary antibody into each well. Incubate at room temperature for 2 hours (or overnight at 4 °C) in the dark with rocking agitation.

3.3 Wash with PBSTx 3x5 minutes, then 3x20 min at room temperature.

4. Mounting

4.1 Relocate the embryos from each well onto a slide(s). Remove the liquid from the slides with a tip of filtering paper.

4.2 Put a drop of anti-fade reagent onto each sample cluster. Keep the slides even in the dark overnight at room temperature.

5 Reading & Imaging.

REGENTS AND BUFFERS NOT PROVIDED

1. E2 buffer: NaCl 15.0 mM, KCl 0.5 mM, $MgSO_4$ 1.0 mM, KH_2PO_4 0.15 mM, Na_2HPO_4 0.05 mM, $CaCl_2$ 1.0 mM, $NaHCO_3$ 0.7 mM. Store at 4°C.
2. Pronase: 2 mg/ml in E2 buffer, store at –20 °C.
3. Paraformaldehyde (PFA) (or Formaldehyde (FA)) 4% in PBS. Store at room temperature.
4. PBSTx: PBS with 0.5% Triton X-100. Store at 4°C.
5. Blocking buffer 1: 0.5% BSA, 5% normal goat serum (NGS), 0.1% DMSO, 0.03% NaN_3 in PBSTx. Store 4°C.
6. Blocking buffer 2: 0.5% BSA, 0.1% DMSO, 0.03% NaN_3 in PBSTx. Store 4°C.

This product is for *in vitro* research use only.