

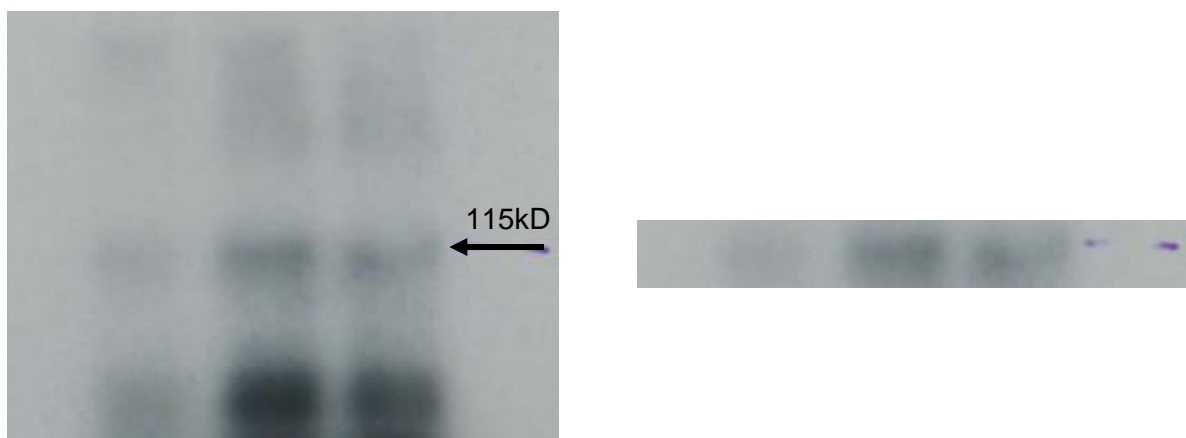
30µg of embryonic day 4 (E4) chick protein was mixed with 10µL of Laemelli solution for 5 minutes at 100°C. The sample was then used for electrophoresis using a stacking gel: upper (4.5% stacking gel) and lower (7.5% SDS PAGE) at 120mV. After the electrophoresis, the gel was transferred onto a methanol-activated membrane for 1.5 hours at 300mA on ice. The membrane was then rinsed for 1 minute in phosphate-buffered saline (PBS) and then blocked for 1 hour in 5% skim milk.

The membrane was incubated at 4°C overnight (in a shaker) with DNMT3a antibody (NOVUS 64B1446) diluted 1:1,000 in 10% Bovine Serum Albumin (BSA) (Fisher Scientific BP1605-100) in PBS.

The membrane was washed in PBS with triton (PBST) 3x for 5 minutes. The membrane was incubated (shaking) at room temperature 2 hours with an anti-mouse HRP secondary antibody (Cell Signaling #7076) diluted in 10% BSA, followed by 4x 10 minute washes in PBST.

The membrane was then exposed to a chemoluminescent reaction (Thermo Scientific #34078) for 20 minutes before exposed to X-ray film.

The DNMT3a band was found to be about 115kD^{1,2}.



1. Trasler, J.M. and La Salle, S. Dynamic expression of DNMT3a and DNMT3b isoforms during male germ cell development in the mouse. *Dev. Biol.* **2006**, 296: 71-82. doi:10.1016/j.ydbio.2006.04.438
2. Novus Biologicals. DNMT3a Antibody (64B1446). http://www.novusbio.com/DNMT3A-Antibody-64B1446_NB120-13888.html#dsTab1 (accessed July 21, 2016).