# NR2F6 (*Homo sapiens*), TCF3 (*Homo sapiens*), ZNF7 (*Homo sapiens*), and ZNF12 (*Homo sapiens*)

Method:
Western Blot Validation

Caption:
Each nuclear protein isolate (50 mcg) was standardized in a solution containing a volume of 2% Halt Protease and Phosphatase Inhibitor Single-Use Cocktail Mixture (Thermo Fisher Scientific), NuPage Sample Reducing Agent 10X, and NuPage LDS Sample Buffer 4X (Thermo Fisher Scientific). After heating the solution for 15 minutes at 90C followed by cooling on ice, the protein samples were loaded onto a NuPage 4-12% Bis-Tris gel (Thermo Fisher Scientific) and separated using a PowerEase 90W system (Thermo Fisher Scientific) running at 150 V for 1 hour. A K562 untagged nuclear isolate was included as a negative control. The protein bands were transferred to a nitrocellulose membrane using the Invitrogen iBlot 2 System (Thermo Fisher Scientific), and blocked overnight at 4C in 5% milk solution with gentle rocking. The membrane was treated with a 1:5000 dilution of monoclonal M2-Peroxidase-conjugated ANTI-FLAG antibody (diluted in 5% BSA solution) (Sigma-Aldrich; cat# A8592) for 1 hour. Following four 5-minute washes with 1X TBST, visualization was attained with the Super Signal West Femto solution kit (Thermo Fisher Scientific) and a MyECL Imager (Thermo Fisher Scientific).
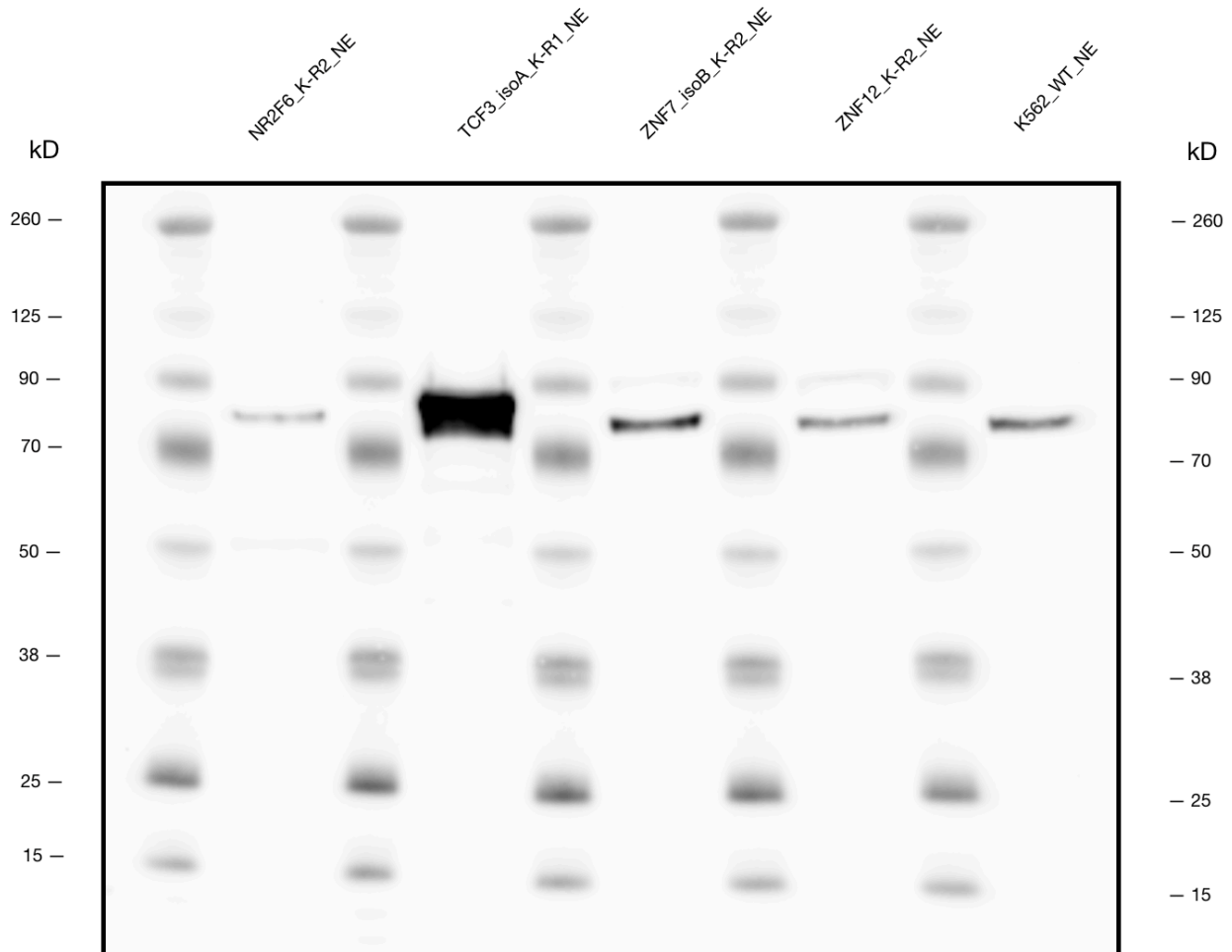
Monoclonal ANTI-FLAG M2-Peroxidase (HRP) antibody produced in mouse

Lane	Loaded Sample	Expected Band Size (kDa)	Comments
1	Ladder	N/A	N/A
2	FLAG-NR2F6_K562 rep 2 (nuclear extract)	46	Faint band near the expected
3	Ladder	N/A	N/A
4	FLAG-TCF3_isoA_K562 rep 1 (nuclear extract)	71	Dark band within 20% from the expected. PTMs: Isopeptide bonding, Methylation, Phosphorylation, and Ubl conjugation
5	Ladder	N/A	N/A
6	FLAG-ZNF7_isoB_K562 rep 2 (nuclear extract)	81	Faint band within 20% of the expected. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
7	Ladder	N/A	N/A
8	FLAG-ZNF12_K562 rep 2 (nuclear extract)	84	Faint band within 20% of the expected. PTMs: Isopeptide bonding and Ubl conjugation
9	Ladder	N/A	N/A
10	Wild-Type K562 (nuclear extract) (negative control)	None	Single non-distinct band

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