

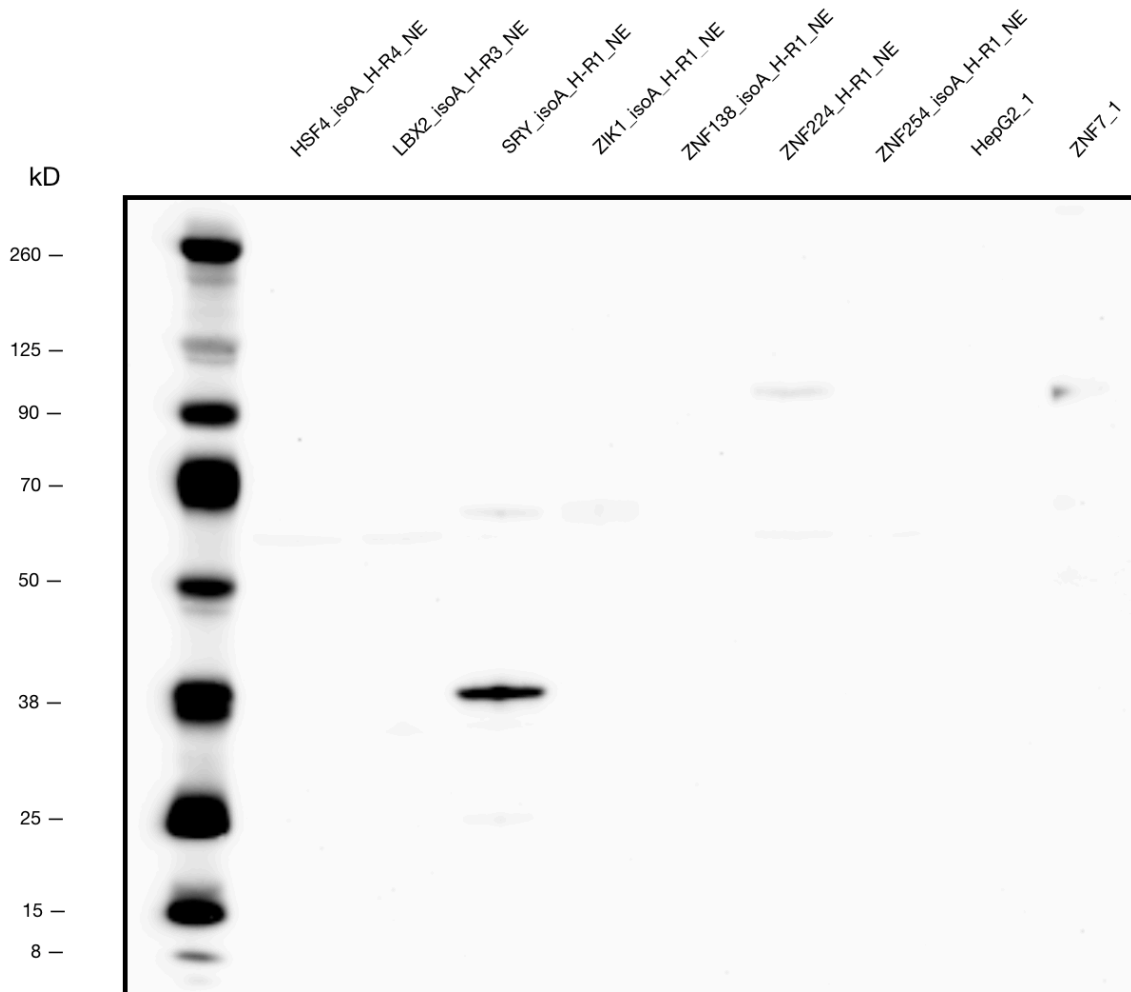
**HSF4 (*Homo sapiens*), LBX2 (*Homo sapiens*), SRY (*Homo sapiens*), ZIK1 (*Homo sapiens*), ZNF138 (*Homo sapiens*), ZNF224 (*Homo sapiens*), and ZNF254 (*Homo sapiens*)**

**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 HepG2 untagged nuclear isolate was included as a negative control, and a ZNF7-tagged nuclear isolate as a positive 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-HSF4_isoA_HepG2 rep 4 (nuclear extract)	56	Single faint band within 20% expected. PTMs: Isopeptide bonding, Phosphorylation, and Ubl conjugation
3	FLAG-LBX2_isoA_HepG2 rep 3 (nuclear extract)	24	Extremely faint band near 60 kDa, further than 20% from the expected
4	FLAG-SRY_isoA_HepG2 rep 1 (nuclear extract)	27	Dark band around 38 kDa. Found comparable western blot with banding near 36 kDa, within 20% from the observed: <a href="https://www.biorbyt.com/sry-antibody-orb225807.html">https://www.biorbyt.com/sry-antibody-orb225807.html</a> . PTMs: Acetylation and ADP-ribosylation
5	FLAG-ZIK1_isoA_HepG2 rep 1 (nuclear extract)	58	Very faint banding within 20% expected
6	FLAG-ZNF138_isoA_HepG2 rep 1 (nuclear extract)	34	No visible banding
7	FLAG-ZNF224_HepG2 rep 1 (nuclear extract)	85	Distinct band around 100 kDa. Found comparable western blot with banding near the same molecular weight as the observed: <a href="https://www.lsbio.com/antibodies/znf224-antibody-aa395-444-wb-western-ls-c116790/119625">https://www.lsbio.com/antibodies/znf224-antibody-aa395-444-wb-western-ls-c116790/119625</a>
8	FLAG-ZNF254_isoA_H-R1_NE (nuclear extract)	80	No visible banding
9	Wild-Type Hep G2 (nuclear extract) (negative control)	None	No visible banding
10	FLAG-ZNF7_1 (nuclear extract) (positive control)	81	Partial band within 20% expected

**Submitted by:**

Mark Mackiewicz and Michael Betti

**Lab:**

Richard Myers, HAIB

**Grant:**

UM1 HG009411

**Download:**