Validation Report #029611

Validation Date: 02/12/14

Summary

Antigen	Low Density Lipoprotein Receptor-Related Protein 2 (LRP2) antibody (Cy3)
Catalog number	ABIN750991
Supplier	Bioss
Supplier catalog number	bs-3909r-cy3
Lot number	YEYY9
Method validated	Immunofluorescence
Laboratory	CaresBio Laboratory
Validation number	29611
Positive Control	MCF7 cells
Negative Control	HeLa cells
Notes	Strong signal was detected in positive control tissues and not in negative control tissues. Note that there was a small amount of signal generated in the negative control sample.

Full Methods

Primary Antibody
- Antigen: Low Density Lipoprotein Receptor-Related Protein 2 (LRP2) antibody (Cy3)
- Catalog number: ABIN750991
- Supplier: Bioss
- Supplier catalog number: bs-3909r-cy3
- Lot number: YEYY9

Isotype Control Antibody
- Antibody: Normal rabbit serum IgG
- Supplier: Santa Cruz Biotechnology
- Catalog number: sc-2338

Secondary Antibody

Used only for the secondary only control, as primary antibody was directly conjugated to Cy3
- Antibody: Cy3 Goat Anti-Rabbit IgG (H+L)
- Supplier: Life Technologies
- Catalog number: A10520

Controls
- Positive control: MCF7, LRP2 positive breast carcinoma cells (specimen known to contain the target protein) from ATCC.
- Negative Control: HeLa, LRP2 negative cervical carcinoma cells (specimen known to not contain the target protein) from http://www.atcc.org/products/all/CCL-2.aspx.
- Isotype Control: Normal rabbit serum IgG (cat # sc-2338) from SantaCruz Biotechnology.
- Secondary antibody only control: MCF7, LRP2 positive breast carcinoma cells incubated with Goat anti-Rabbit CY3 secondary antibody only.

Protocol
- MCF7 and HeLa cell lines were grown directly on coverslips and fixed with 4% formaldehyde in PBS for 15 min at room temperature (RT).
- Fixed cells were rinsed three times in PBS for 5 min each at RT.
- Cells were blocked in 1X PBS/1% BSA/0.3% Triton™ X-100 to block unspecific binding of the antibodies for 60 min at RT.
- Cells were incubated with fluorophore conjugated primary antibody diluted 1:250 and 1:100 in 1X PBS/1% BSA/0.3% Triton™ X-100 overnight at 4°C.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Coverslips were mounted on slides with ProLong® Gold Antifade Reagent with DAPI.

Isotype control staining: All the steps are done same as previously described until cells were blocked in 1X PBS/1% BSA/0.3% Triton™ X-100 to block unspecific binding of the antibodies for 60 min at RT.
- Cells were incubated with 10% normal rabbit serum overnight at 4°C.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Cells were incubated with goat anti rabbit CY3 conjugated secondary antibody for 60 min in dark at RT.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Coverslips were mounted on slides with ProLong® Gold Antifade Reagent with DAPI.

Secondary only staining: All the steps are done same as previously described until cells were blocked in 1X PBS/1% BSA/0.3% Triton™ X-100 overnight at 4°C.
- Cells were incubated with goat anti rabbit CY3 conjugated secondary antibody for 60 min in dark at RT.
- Cells were rinsed three times in PBS for 5 min each at RT.
- Coverslips were mounted on slides with ProLong® Gold Antifade Reagent with DAPI.
- Stained cells were imaged with a Nikon C2+ confocal microscope.

Experimental Notes

We did the first set of experiment with 1:250 dilution of the primary antibody but have observed lower level of expression of LRP2 in MCF7 (positive) cells (Figure 2). We repeated experiment with higher concentration of the

primary antibody (1:100) and the expression level increased as shown in Figure 1. We have noticed lower level of expression of LRP2 in HeLa (negative) cells (Figure 3). We have used rabbit normal serum for isotype control to match the species with primary antibody (rabbit) and also we used bovine albumin serum (BSA) as blocking and antibody diluent buffer to reduce species cross reactivity.

Figures

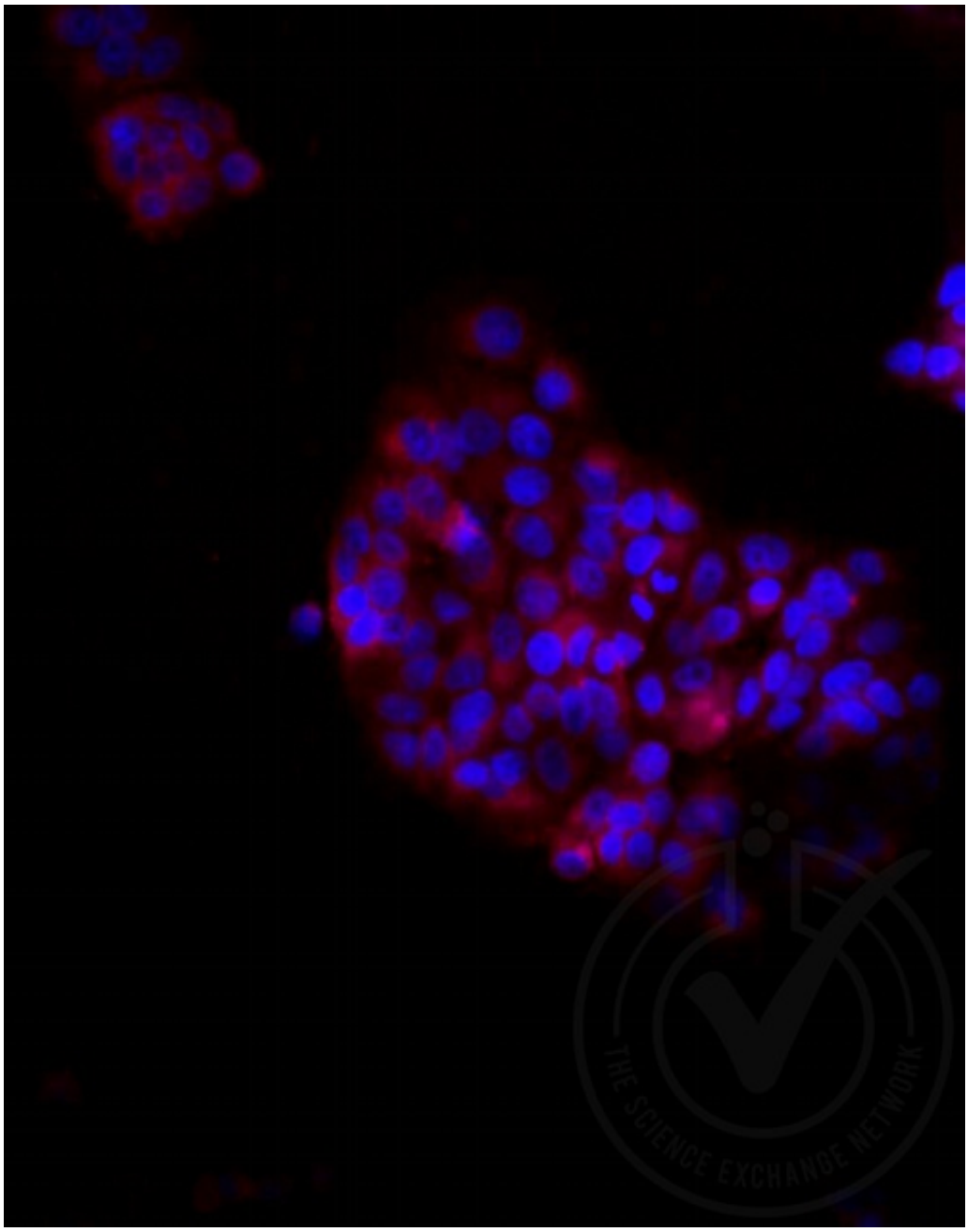

Figure 1: MCF7 cells stained at 1:250 with LRP2 (red) and a nuclear counterstain (blue).

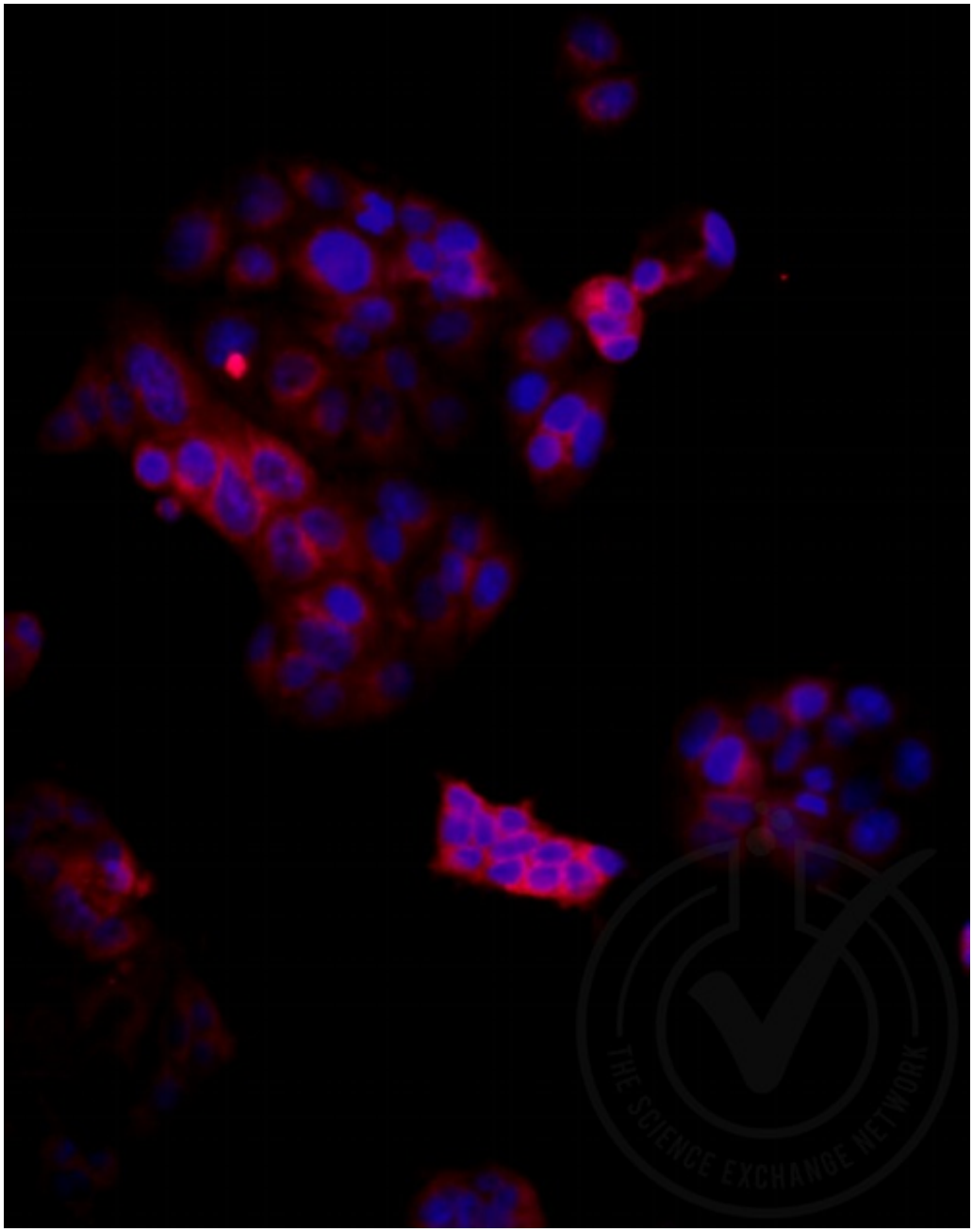

Figure 2: MCF7 cells stained at 1:100 with LRP2 (red) and a nuclear counterstain (blue).

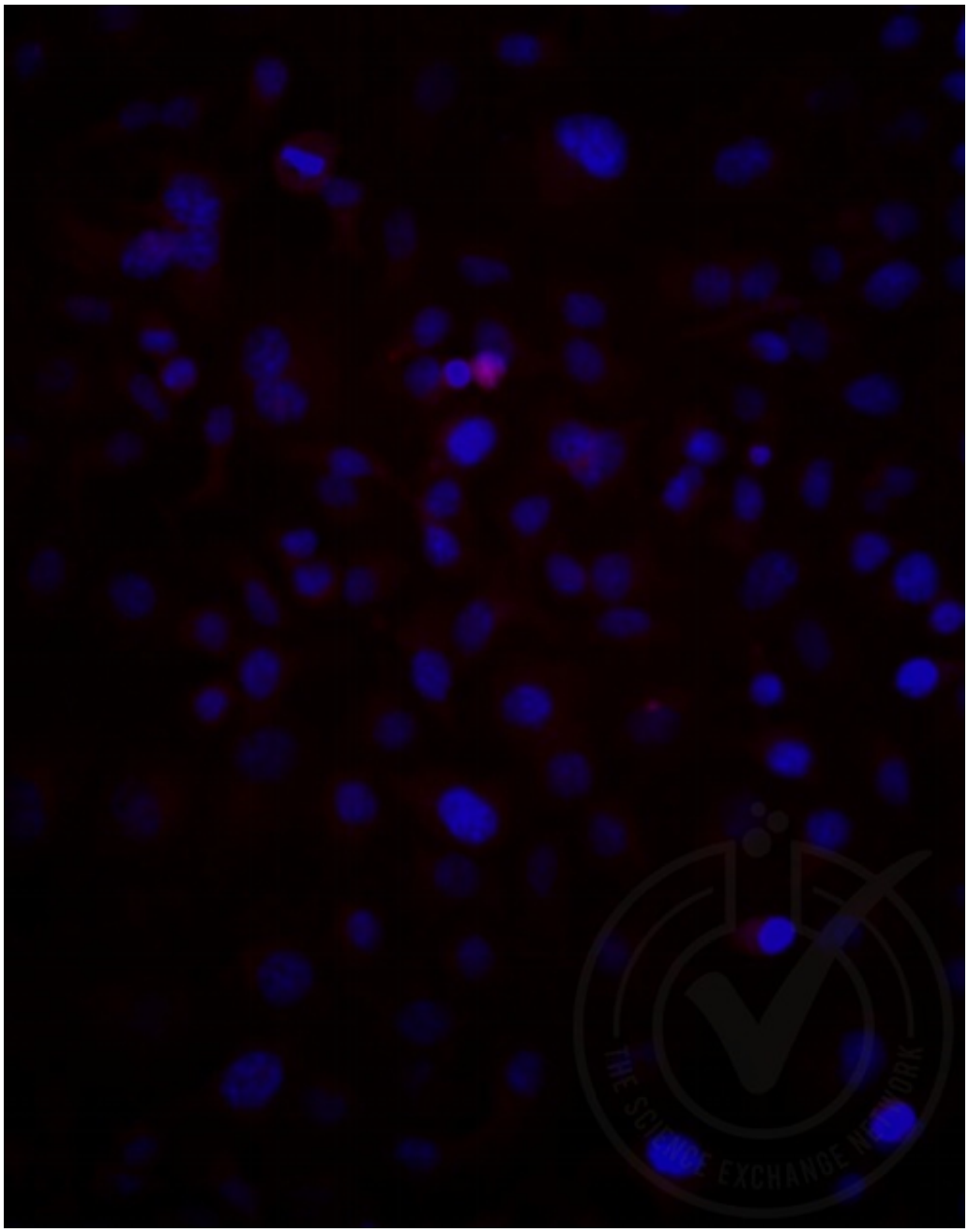

Figure 3: HeLa cells stained with LRP2 (red) and a nuclear counterstain (blue).

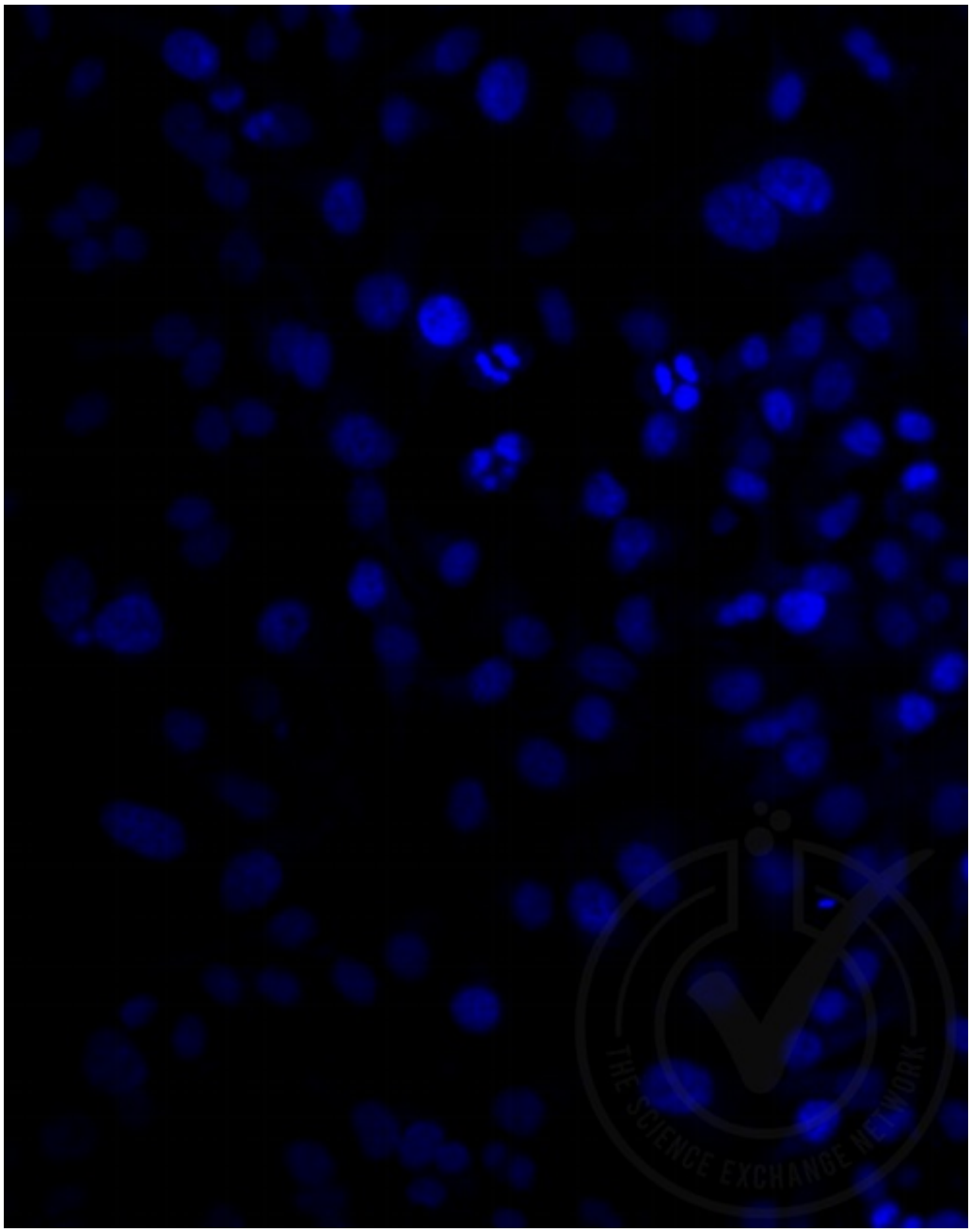

Figure 4: MCF7 cells stained isotype control (red) and a nuclear counterstain (blue).

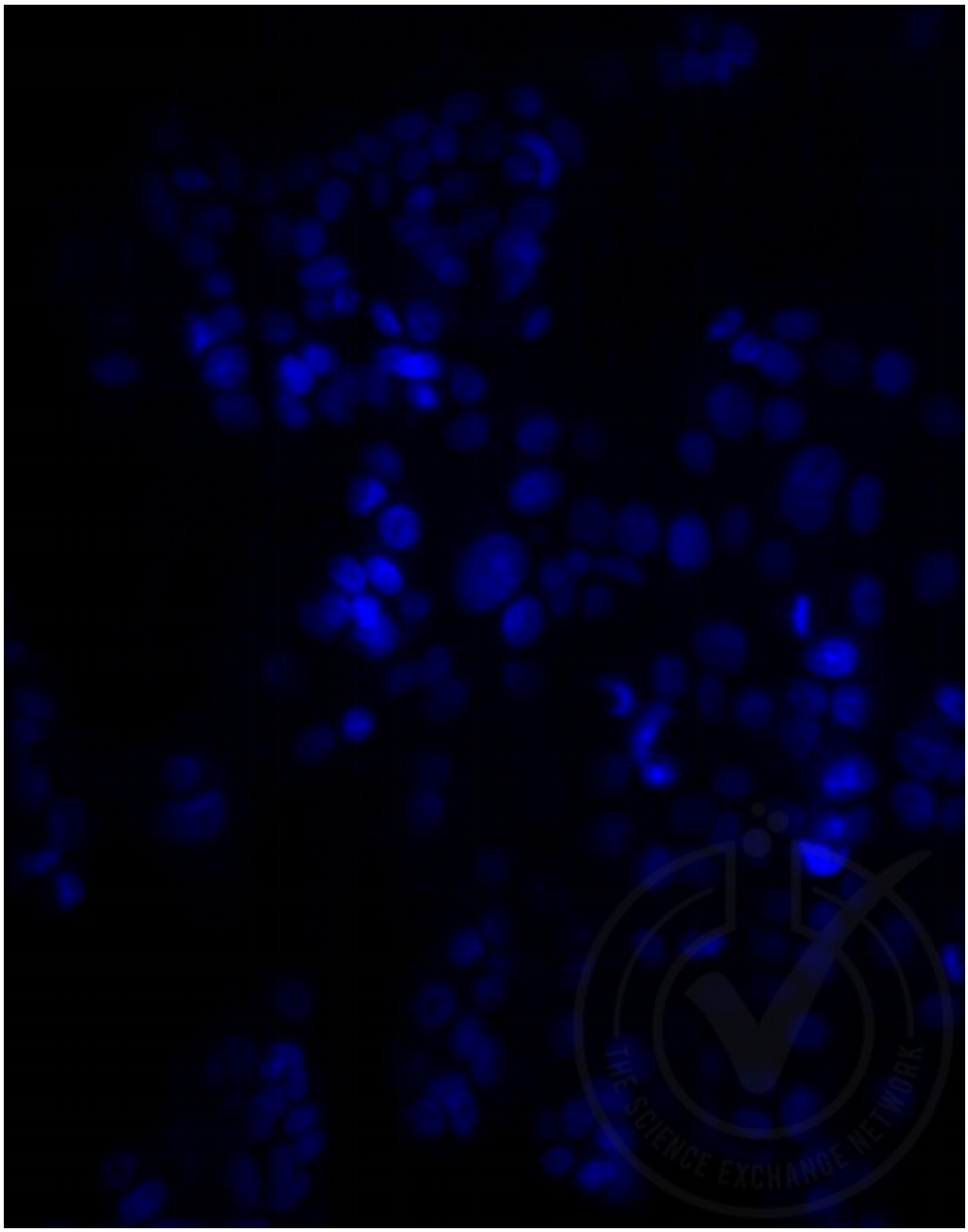

Figure 1: MCF7 cells with only a secondary antibody (red) and a nuclear counterstain (blue).