Rosen Lab immunostaining conditions

Antibody	Host	Source	Cat #	Antigen Retrieval	Block	Diluent	Ab Dilution	2° antibody
Androgen receptor	rabbit polyclonal	Upstate Biotech	06-680	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:500	IHC 1:250 biotinylated goat α-rabbit
Aquaporin 5	rabbit polyclonal	Alpha Diagnostics	AQP51-A	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:500 IF 1:50	IHC 1:250 biotinylated goat α-rabbit IF 1:1000 goat α-rabbit Texas Red
BrdU	FITC-conjugated mouse monoclonal	BD Biosciences	347583	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:5-1:10	no secondary needed (already FITC conjugated)
Caspase 3, cleaved (Asp 175)	rabbit polyclonal	Cell Signaling	9661	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:1000	IHC 1:250 biotinylated goat α-rabbit
C/EBPß	mouse monoclonal	Santa Cruz	sc-7962 (H7)	10 mM Citrate pH 6 20 min microwave	MOM block	MOM diluent	IHC 1:100	IHC 1:250 biotinylated rabbit α-mouse
cyclin D1	rabbit polyclonal	NeoMarkers	RB-212-P (Ab-4)	None	5% milk in PBST	1% milk in PBS	IHC 1:250	IHC 1:250 biotinylated goat α-rabbit
E-Cadherin	rat monoclonal	Zymed	13-900	10 mM Citrate pH 6 20 min microwave	MOM block	MOM diluent	IHC 1:100 IF: 1:1000	IHC 1:250 biotinylated goat α-rat IF 1:1000 goat α-rat Texas Red
phospho ERK (Thr202/Tyr204)	rabbit polyclonal	Cell Signaling	9201	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:100	IHC 1:250 biotinylated goat α-rabbit
phospho ERM	rabbit polyclonal	Cell Signaling	3141	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:10,000	IHC 1:250 biotinylated goat α-rabbit
Estrogen receptor alpha	rabbit polyclonal	Santa Cruz	sc-542 (MC-20)	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:200	IHC 1:250 biotinylated goat α-rabbit IF 1:1000 goat α-rabbit Texas Red
F4/80 (macrophage)	rat monoclonal	Caltag	RM2900	None	MOM block	MOM diluent	IHC 1:10,000 IF 1:500	IHC 1:250 biotinylated goat α-rat
phospho-Histone H1	rabbit polyclonal	Upstate Biotech	06-597	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:2,000 IF 1:100	IHC 1:250 biotinylated goat α-rabbit IF 1:1000 goat α-rabbit Texas Red
phospho-Histone H3 (ser10)	rabbit polyclonal	Upstate Biotech	06-570	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:5,000 IF 1:300	IHC 1:250 biotinylated goat α-rabbit IF 1:1000 goat α-rabbit Texas Red
IRS-1	rabbit polyclonal	Upstate Biotech	06-248	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:400	IF 1:1000 goat α-rabbit Texas Red
IRS-2	rabbit polyclonal	Upstate Biotech	06-506	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:400	IF 1:1000 goat α-rabbit Texas Red
Keratin 5 (myoepithelial)	rabbit polyclonal	Covance	PRB-160P	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:5,000 IHC 1:10,000	IF 1:1000 goat α-rabbit Texas Red IHC 1:250 biotinylated goat α-rabbit
Keratin 6	rabbit polyclonal	Covance	PRB-169P	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:200 IHC 1:5,000	IF 1:1000 goat α-rabbit Texas Red IHC 1:250 biotinylated goat α-rabbit
Keratin 8 (luminal epithelial)	rat monoclonal	Developmental Studies Hybridoma Bank (Univ of Iowa)	TROMA-1	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20 (or MOM kit)	5% BSA/0.5% Tween-20 (or MOM kit)	IF 1:250 IHC 1:5000	IF 1:1000 donkey α-rat Texas Red IHC 1:250 biotinylated goat α-rat
Keratin 14 (myoepithelial)	rabbit polyclonal	Covance	PRB-155P	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:400	IF 1:1000 goat α-rabbit Texas Red IHC 1:250 biotinylated goat α-rabbit
Keratin 19 (luminal epithelial)	mouse monoclonal	GeneTex	GTX27755 (BA-17)	10 mM Citrate pH 6 20 min microwave	MOM block	MOM diluent	IF 1:5,000 IHC 1:10,000	IF 1:1000 rabbit α-mouse Texas Red
Ki67	goat polyclonal	Santa Cruz	sc-7846 (M-19)	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:5,000	IHC 1:250 biotinylated donkey α-goat
NKCC1	rabbit polyclonal	Jim Turner, NIH	n/a	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:50	IF 1:1000 goat α-rabbit Texas Red
p21	mouse monoclonal	DAKO	M7202 (SX-118)	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:200	IHC 1:250 biotinylated goat α-rabbit
p27	rabbit polyclonal	NeoMarkers Santa Cruz	RB-006-P1 (Ab2) sc-776 (M-197)	None	5% milk in PBST	1% milk in PBS	IF 1:200	IHC 1:250 biotinylated goat α-rabbit
phospho-p27 (Thr187)	rabbit polyclonal	Zymed	71-7700	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IHC 1:100	IF 1:1000 goat α-rabbit Texas Red
p63	mouse monoclonal	NeoMarkers	MS-1081-P (Ab1)	10 mM Citrate pH 6 20 min microwave	MOM block	MOM diluent	IHC 1:500	IHC 1:250 biotinylated rabbit α-mouse
Progesterone receptor	rabbit polyclonal	DAKO	A0098	10 mM Citrate pH 6 20 min microwave	5% BSA/0.5% Tween-20	5% BSA/0.5% Tween-20	IF 1:50 IHC 1:1000	IF 1:1000 goat α-rabbit Texas Red IHC 1:250 biotinylated goat α-rabbit
RbAp46	goat polyclonal	Santa Cruz	sc-8272 (N-19)	None	5% milk in PBST	1% milk in PBS	IHC 1:1000	IHC 1:250 biotinlyated donkey α-goat
Sca-1	rat monoclonal	Becton-Dickinson	557403	None	5% milk in PBST	1% milk in PBS	IHC 1:100	IHC 1:250 biotinylated goat α-rat
Smooth muscle actin (clone A14)	mouse monoclonal	Sigma	A2547	10 mM Citrate pH 6 20 min microwave	MOM block	MOM diluent	IF 1:250	IF 1:1000 rabbit α-mouse Texas Red
ZO-1	rat monoclonal	Zymed	33-9100	10 mM Citrate pH 6 20 min microwave	MOM block	MOM diluent	IF 1:50	IF 1:1000 donkey α-rat Texas Red

Notes regarding immunostaining

These conditions have been optimized on mouse mammary gland sections and have not been tested on other tissues. Tissues were normally fixed 2 hours in 4% paraformaldehyde before processing and embedding in paraffin. Five micron sections were cut and placed on charged microscope slides. (Probe-On Plus, Fisher #15-188-52)

IHC = immunohistochemistry IF = immunofluorescence

Sources of secondary antibodies:

1. biotinylated goat α-rabbit (Oncogene #OS03B-200UG)
2. biotinylated rabbit α-mouse (Oncogene #OS02B-200UG)
3. biotinylated donkey α-goat (Molecular Probes #D-20698)
4. biotinylated goat α-rat (Vector #BA-9400)

5. goat α-rabbit Texas Red (Molecular Probes #T-6391)
6. goat α-rabbit Alexa 488 [green] (Molecular Probes #A-11008)
7. goat α-mouse Texas Red (Molecular Probes #T-6390)
8. goat α-mouse Alexa 488 [green] (Molecular Probes #A-11001)
9. goat α-rat Texas Red (Molecular Probes #T-6392)
10. goat α-rat Alexa 488 [green] (Molecular Probes #A-11006)

Other Reagents:

1. BSA = Bovine Serum Albumin, Fraction V
   Sigma #A-7906
2. Milk = Carnation Instant Milk Powder
   5% = 1 gram in 20 ml PBST
3. PBS = phosphate buffered saline
4. PBST = phosphate buffered saline + 0.1% Triton X-100
5. Citrate = 100 mM citric acid, pH 6 stock solution
   working solution = 10 mM

Kits used for immunohistochemistry:

1. Vectastain ABC Elite Kit (Vector #PK-6100)
2. MOM (mouse on mouse) Kit (Vector #BMK-2202)
3. DAB (brown) peroxidase staining kit (Vector #SK-4100)
4. VIP (purple) peroxidase staining kit (Vector #SK-4600)

NOTE: Immunohistochemistry, as performed by our protocols, is about 20x more sensitive than immunofluorescence.

So, if an antibody is used at 1:100 for IF, a good starting point for IHC would be 20-times more dilute, or 1:2000. The reverse is also true: an antibody used at 1:10,000 for IHC would need to be diluted ~1:500 for IF.