Comparison of Immunohistochemical Staining of Signaling Markers in Frozen and Paraffin-Embedded Tissues.

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Introduction

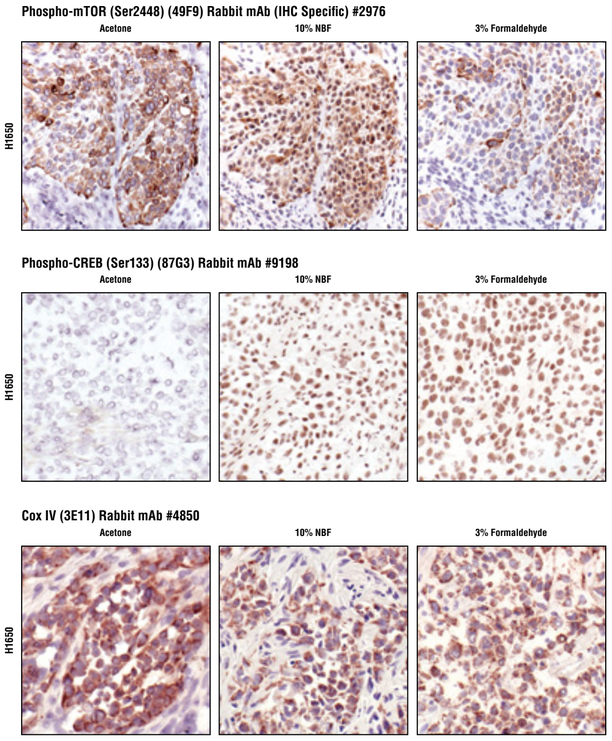
Tissue preparation for immunohistochemical staining may be done by paraffin embedding or freezing. To date, the great majority of IHC studies using phospho-specific antibodies have used formalin-fixed paraffin embedded tissues; however, a limited number of studies have suggested that phospho-specific epitopes may be detected in frozen sections as well. In this study we analyzed a broad panel of phospho-specific and total antibodies directed against various signaling markers on matched fresh frozen or formalin-fixed paraffin embedded xenograft tumors. The results suggest that phospho-epitopes survive fixation and tissue processing, and are not necessarily degraded or dephosphorylated as fixative penetrates. Staining in paraffin embedded samples matched or was superior to that seen in frozen tissues, though antigen retrieval was often required, including for p-EGFR, p-CREB, p-S6 and p-Histone H3. Determining the optimal fixative for frozen sections was found to be imperative for many antibodies, for example p-erk and p-mTOR. These results will be useful for future studies that are conducted on frozen tissues.

Methods

Xenografts were initiated using 5-10x10^6 cells in 50% Matrigel™ injected subcutaneously into NCR/nu mice. Upon harvest tumors were divided into multiple pieces. Tissues were either snap frozen in liquid nitrogen and embedded in OCT or fixed for 24 hours in 10% neutral buffered formalin, then processed and embedded in paraffin per standard methods. Frozen sections were cut at 7-8 µm and paraffin sections were cut at 4-5 µm. Immunohistochemical analysis on frozen and paraffin sections was performed according to standard CST protocols, with primary antibody incubations overnight at 4°C using the same recommended dilution for frozen and paraffin staining. All antibodies were from CST.

Antibody	Xenograft	Frozen Sections				Paraffin Sections	
		Acetone	10% NBF	3% Formaldehyde	3% Formaldehyde/MeOH	With Retrieval	Without Retrieval
EGF Receptor Antibody #2232	H1650	+	++	+++	+++	+++	+/-
Phospho-EGF Receptor (Tyr845) Antibody #2231	H1650	-	-	-	-	++	-
Phospho-EGF Receptor (Tyr1173) (53A5) Rabbit mAb #4407	H1650	-	-	-	-	++	-
Phospho-HER2/ErbB2 (Tyr1221/1222) (6B12) Rabbit mAb #2243	HCC827	-	++	NT	++	+++	-
Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb #9145	H1650	-	+++	++	+++	++	-
Phospho-Akt (Ser473) (736E11) Rabbit mAb (IHC Specific) #3787	U-87MG	-	-	+++	+++	+++	++
Phospho-Akt (Thr308) (244F9) Rabbit mAb #4056	H1650	-	-	++	NT	++	-
Phospho-p44/42MAPK (Thr202/Tyr204) (20G11) Rabbit mAb (IHC Preferred) #4376	H1650	-	+++	++	++	+++	+
Phospho-S6 Ribosomal Protein (Ser235/236) (91B2) Rabbit mAb (IHC Preferred) #4857	H1650	-	++	++	++	+++	++
Phospho-mTOR (Ser2448) (49F9) Rabbit mAb (IHC Specific) #2976	H1650	+++	-	NT	++	+++	-
Phospho-p38 MAPK (Thr180/Tyr182) (12F8) Rabbit mAb (IHC Preferred) #4631	H1650	-	+++	NT	++	+++	+/-
Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251	H1650	-	++	NT	+/-	++	-
Phospho-BAD (Ser112) (40A9) Rabbit mAb #5284	H1650	-	-	-	NT	+	-
Phospho-HSP27 (Ser82) Antibody #2401	H1650	-	+++	+	NT	++	+
Phospho-Chk2 (Thr68) (80F5) Rabbit mAb #2584	H1650	-	-	-	NT	+++	-
Phospho-IKKalpha/beta (Ser176/180) (16A6) Rabbit mAb #2697	H1650	+	+++	++	+++	++	+/-
Bcl-xL (54H6) Rabbit mAb #2764	H1650	-	++	+	-	+++	+/-
c-Jun (60A8) Rabbit mAb #9165	H1650	+	+++	+++	NT	+++	+/-
Phospho-c-Jun (Ser63) (54B3) Rabbit mAb #2361	H1650	-	+/-	-	NT	+/-	-
Phospho-CREB (Ser133) (87G3) Rabbit mAb #9198	H1650	-	+++	NT	+++	+++	-
Phospho-Histone H3 (Ser10) Antibody #9701	H1650	+	+++	++	++	+++	-
Cox IV (3E11) Rabbit mAb #4850	H1650	+++	+++	+++	NT	+++	-
Survivin (71G4) Rabbit mAb #2808	H1650	++	+++	++	NT	+++	+
Cleaved Caspase-3 (Asp175) Antibody #9661	H1650	-	+	+++	NT	+++	+
β-Actin (13E5) Rabbit mAb #4970	H1650	-	+++	++	-	+++	++
α/β-Tubulin Antibody #2148	H1650	++	+/-	+++	NT	+++	++
Caveolin Antibody #3238	H1650	++	++	+++	NT	+++	-

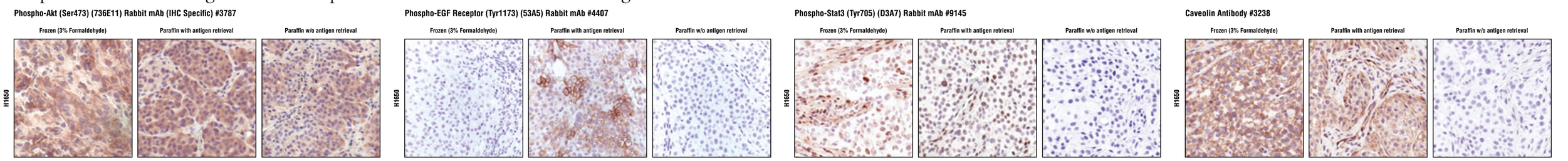
Fixation optimization for frozen sections.



Conclusion

- Optimization of fixation conditions for frozen sections is necessary to obtain ideal staining with phospho-specific and total antibodies.
- Phospho epitopes are not necessarily destroyed as a matter of course through fixation and paraffin-embedding.
- Staining achieved with two anti-p-EGFR antibodies was superior in formalin-fixed paraffin-embedded xenograft samples.
- Staining with phospho-specific antibodies can be achieved in frozen samples without unmasking epitopes, as is often required for paraffin-embedded samples.

Comparison of IHC staining on frozen and paraffin sections with and without antigen retrieval.



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