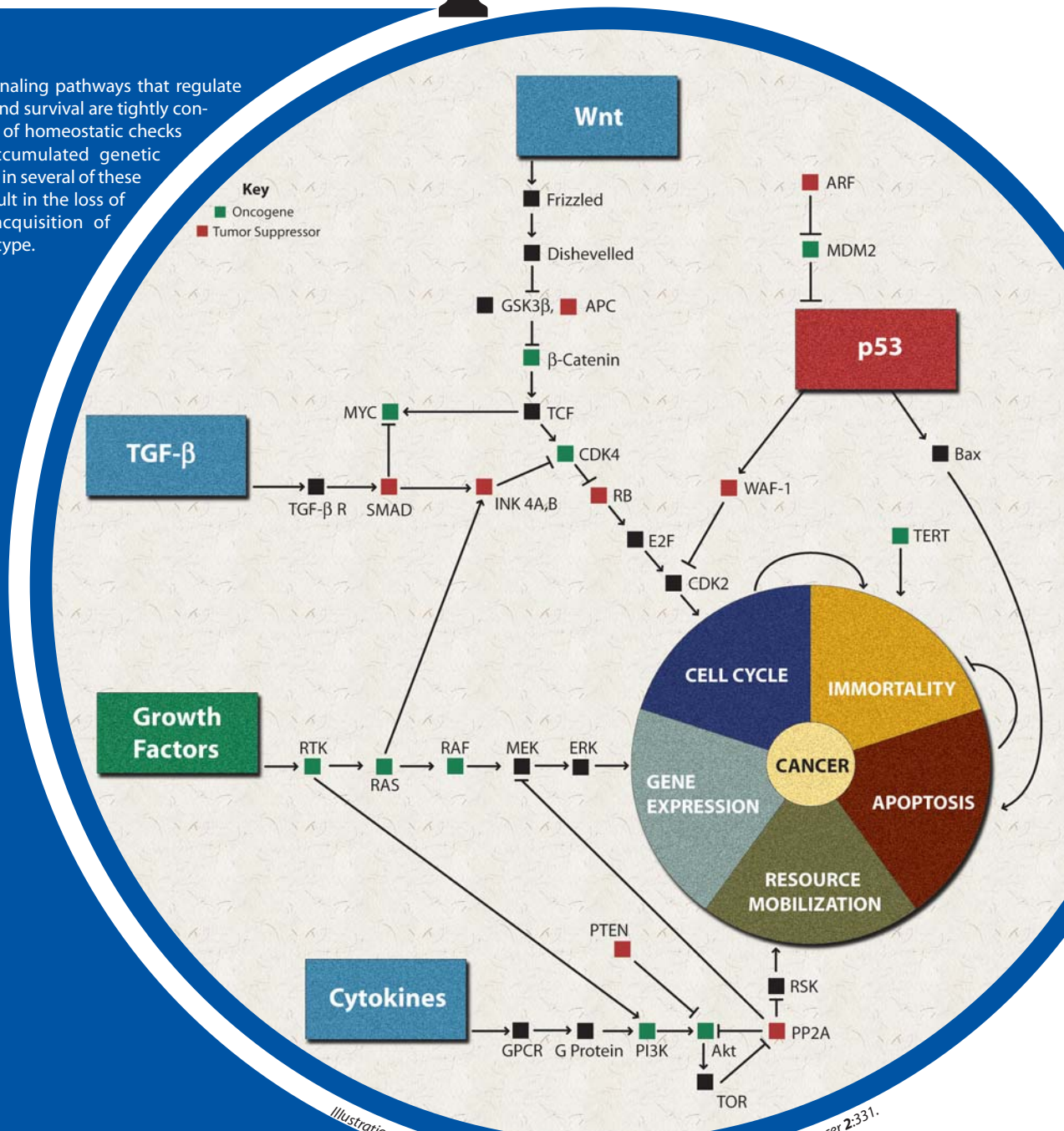


# In Scope

**Figure Legend**

The molecular signaling pathways that regulate cell proliferation and survival are tightly controlled by a series of homeostatic checks and balances. Accumulated genetic mutations, usually in several of these pathways, can result in the loss of regulation and acquisition of the cancer phenotype.



*Illustration has been adapted from Hahn, W.C. & R.A. Weinberg (2002) Nat. Rev. Cancer 2:331.*

**Inside**

p53: Key Regulator of the Cellular Stress Response .....2  
 Frizzled-Related Proteins in Carcinogenesis .....3  
 ERK Activation and Growth Factor Signaling .....6  
 Human Tissue Kallikreins as Cancer Markers .....7

March 2003



everything cytokine & beyond

## Apoptosis and DNA Damage & Repair

In addition to mutations in oncogenes and tumor suppressors, alterations in DNA repair genes that are involved in maintaining genomic stability are also a clear cause of tumor development. DNA is subject to constant insult from endogenous and exogenous agents. While this damage may result in immediate induction of apoptosis, in most cases cells will attempt to repair the damage. DNA repair processes work in concert with the cell cycle and apoptosis to maintain genomic fidelity and integrity and to counteract the deleterious effects of DNA damage. Cells usually repair the damage efficiently, but occasionally repair mechanisms fail, leading to altered cellular function and mutations. This failure may induce apoptosis, however, the new mutations may also prevent apoptosis and lead to the development of a cancerous phenotype.

A greater understanding of the links between apoptosis, DNA damage and repair, and cancer will lead to the development of more effective cancer prevention and treatment strategies. R&D Systems offers an extensive line of products to support research into these overlapping research fields. Apoptosis and DNA damage and repair kits, antibodies, enzymes and substrates are available to characterize the damage and can be used alone or in conjunction with other methodologies.

**For a complete list of apoptosis detection kits or DNA damage and repair products, please visit our web site at [www.RnDSystems.com](http://www.RnDSystems.com), click on Catalog and select Apoptosis Kits & Reagents or DNA Damage & Repair in the Product Group drop-down.**

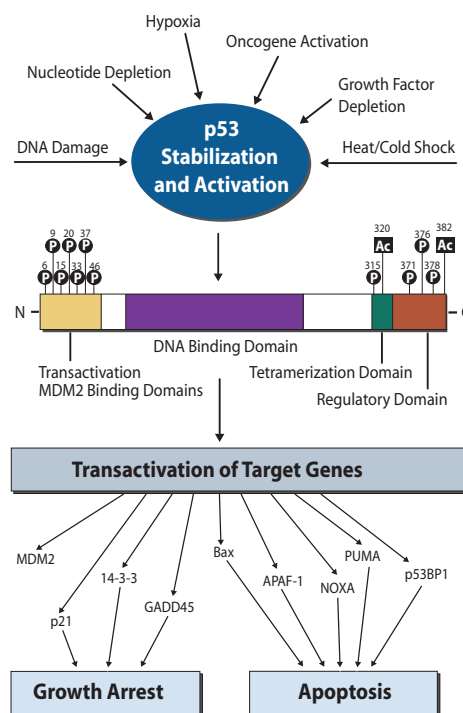
# p53: Key Regulator of the Cellular Stress Response

The p53 tumor suppressor protein plays a pivotal role in the cellular response to an array of stress stimuli including DNA damage, nucleotide depletion, hypoxia, oncogenic activation, growth factor depletion, and temperature shock.<sup>1</sup> In response to these stimuli, p53 may induce cell cycle arrest. This allows either for time to repair damaged DNA prior to cell division, or to initiate apoptosis, which protects the organism by eliminating the defective cell. The relevance of p53 tumor suppressor function to human health is underscored by the observation that greater than half of all human cancers contain p53 inactivating mutations.<sup>2</sup> Furthermore, tumors that retain wild-type p53 protein often have defects in other factors that influence p53 stability and/or activity.<sup>3</sup>

p53 is a tetrameric, sequence-specific transcription factor that is stabilized and activated in response to cellular stress.<sup>1</sup> The activity of p53 is regulated primarily through post-translational modification of the protein.<sup>4</sup> In normally growing cells, p53 levels are low due to interaction with its negative regulator, MDM2, which targets p53 for nuclear export and proteasome-mediated degradation.<sup>5</sup> Cellular stress induces p53 phosphorylation at multiple N- and C-terminal sites. Phosphorylation of serine 20 (S20) blocks p53/MDM2 interaction, resulting in p53 stabilization and nuclear accumulation.<sup>6,7</sup> Phosphorylation of S15 enhances the transcriptional activity of p53.<sup>8</sup> Lastly, phosphorylation at S46 appears to be critical in the induction of p53-mediated apoptosis.<sup>9</sup> These and other phosphorylation and acetylation events influence DNA binding specificity, self-multimerization, protein-protein interaction, and transcriptional activation, thereby allowing p53 to integrate an appropriate cellular response to a variety of stress signals (see figure 1).<sup>10</sup>

The identification of transcriptional targets has been critical in elucidating the mechanisms by which p53 mediates cell cycle arrest or

apoptosis. More than 100 genes are regulated in a p53-dependent manner, many of which have important roles in cellular growth arrest or apoptosis.<sup>11</sup> Cyclin-dependent kinase inhibitor p21 has been demonstrated to play a major role in p53-mediated G1 arrest, while induction of 14-3-3 $\sigma$  and GADD45 contribute to arrest in G2 phase of the cell cycle.<sup>12,13</sup> Examples of transcriptional targets that mediate p53-induced apoptosis include the genes for Bax, APAF-1, NOXA, PUMA, and p53AIP1.<sup>14</sup>



**Figure 1.** The p53 protein undergoes stabilization and activation in response to a variety of stress stimuli. Highlighted in the diagram are the functional domains of p53, including phosphorylation (circled P) and acetylation (boxed Ac) sites implicated in regulation of p53 activity. Activation leads to the upregulation of several genes that may contribute to p53-mediated growth arrest or apoptosis.<sup>15</sup> [Note: illustration has been adapted from reference 15.]

## References

1. Llungman, M. (2000) *Neoplasia* **2**:208.
2. Hainaut, P. & M. Hollstein (2000) *Adv. Cancer Res.* **77**:81.
3. Vogelstein, B. *et al.* (2000) *Nature* **408**:307.
4. Appella, E. & C.W. Anderson (2000) *Pathol. Biol.* **48**:227.
5. Alarcon-Vargas, D. & Z. Ronai (2002) *Carcinogenesis* **23**:541.
6. Chehab, N.H. *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96**:13777.
7. Unger, T. *et al.* (1999) *EMBO J.* **18**:1805.
8. Dumaz, N. & D.W. Meek (1999) *EMBO J.* **18**:7002.
9. Oda, K. *et al.* (2000) *Cell* **102**:849.
10. Haupt, Y. *et al.* (2003) *Oncogene* **21**:8223.
11. Zhao, R. *et al.* (2000) *Genes Dev.* **14**:981.
12. Harper, J. *et al.* (1994) *Cell* **75**:805.
13. Sionov R.V. & Y. Haupt (1999) *Oncogene* **18**:6145.
14. Bargonetti, J. & J.J. Manfredi (2002) *Curr. Opin. Oncol.* **14**:86.
15. Stewart, Z.A. & J.A. Pietsenpol (2001) *Chem. Res. Toxicol.* **14**:243.

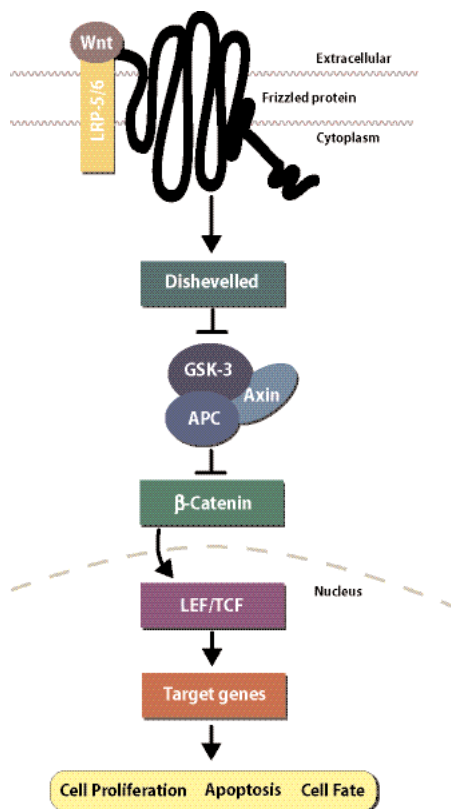
# Frizzled-Related Proteins in Carcinogenesis

Wnt proteins are secreted, glycosylated ligands that play critical roles in embryonic development for a variety of organisms. The canonical Wnt signaling pathway involves binding to receptors of the frizzled family, followed by activation of the cytoplasmic protein dishevelled (Dsh) and disassembly of the APC/axin/GSK protein complex responsible for targeting  $\beta$ -catenin for degradation. Accumulating  $\beta$ -catenin can then enter the nucleus and activate transcription.<sup>1</sup> Mutations in this pathway have been well documented in colorectal cancers.<sup>2,3</sup> In addition to tight control inside the cell, Wnt signaling is also regulated extracellularly. The secreted frizzled-related proteins (sFRPs) were among the first Wnt antagonists to be identified and are implicated in the development of many tissue types. These proteins have significant similarity to the extracellular cysteine rich domain (CRD) of frizzled, suggesting that they interact with Wnts or frizzleds to inhibit signaling.<sup>4</sup>

sFRPs can be classified as tumor suppressors. In breast and cervical tissues, sFRP-1 expression is inversely correlated with malignancy. It is detected in normal breast epithelium, breast epithelial cell lines and benign breast tumors, but is undetectable in 78% of breast carcinomas.<sup>5,6</sup> sFRP-1 is present in normal cervical tissues, but nearly absent in cervical cancer tissues and derived cell lines.<sup>7</sup> sFRP-4 (DDC-4, Frp-AP) is also implicated in breast cancer, it is significantly downregulated in tumors as compared with normal adjacent ductal cells.<sup>8</sup> Interestingly, although levels are lower in breast cancer compared to normal tissues, sFRP-4 expression steadily rises with an increase in tumor grade suggesting a complex role in tumor biology.<sup>8</sup>

sFRPs have a dynamic role in malignant brain tumors. sFRP-1 and -2 are expressed by a majority of malignant glioma cell lines. Ectopic expression of these sFRPs in glioma cells results in increased clonogenicity and enhanced resistance to serum starvation,<sup>9</sup> indicating that sFRPs do not act as tumor suppressors in all cell types. sFRP-2, and to a lesser extent sFRP-1, inhibit glioma cell migration and decrease expression of matrix metalloproteinase 2 (MMP-2).<sup>9</sup> Thus, sFRPs may be involved in

growth promotion, but do not likely enhance the invasive capabilities of glioma cells. Additionally, sFRP-1 induces strong tumor vascularity in grafted gliomas resulting in enlarged, longer, and more mature vessels.<sup>10</sup> In fact, sFRP-1 can induce angiogenesis in a variety of *in vivo* models and may point toward yet another role for the Wnt pathway in cancer.<sup>10</sup>



**Figure 1.** Wnt binding to the frizzled receptor initiates a signal transduction pathway that regulates the levels of intracellular  $\beta$ -catenin. In the absence of Wnt,  $\beta$ -catenin is associated with a multi-protein (APC/axin/GSK3 $\beta$ ) complex where it is ubiquitinated and targeted for degradation. Wnt binding leads to phosphorylation of dishevelled (Dsh) resulting in disassembly of the destruction complex and accumulation of  $\beta$ -catenin in the cytoplasm and nucleus. Wnt signaling is inhibited by secreted Frizzled-related proteins (sFRPs). [Note: illustration has been adapted from Moon, R.T. *et al.* (2002) *Science* **296**:1644.]

## References

1. Miller, J. (2002) *Genome Biol.* **3**:3001.
2. Smalley, M. & T. Dale (1999) *Cancer Metastasis Rev.* **18**:215.
3. Polakis, P. (2000) *Genes Dev.* **14**:1837.
4. Jones, S. & C. Jomary (2002) *Bioessays* **24**:811.
5. Ugolini, F. *et al.* (1999) *Oncogene* **18**:1903.

6. Ugolini, F. *et al.* (2001) *Oncogene* **20**:5810.
7. Ko, J. *et al.* (2002) *Exp. Cell Res.* **280**:280.
8. Wong, S. *et al.* (2002) *J. Pathol.* **196**:145.
9. Roth, W. *et al.* (2000) *Oncogene* **19**:4210.
10. Dufourcq, P. *et al.* (2002) *Circulation* **106**:3097.

## Chemokines & Cancer

Tumor development and growth is a highly complex process involving abnormal gene expression that ultimately leads to cellular transformation, growth, angiogenesis, and metastasis. While the intimate involvement of various cytokines has long since been confirmed, chemokines were only recently implicated in these processes as well as in homing. For example, GRO $\alpha$ /CXCL1 and its mouse homologue MIP-2, IL-8/CXCL8, and RANTES/CCL5 all play roles in melanoma growth and progression when chronically expressed. Additionally, metastasis of melanoma to certain organs correlates with the expression of specific chemokine receptors, such as CXCR4, CCR7, and CCR10. Chemokines also upregulate production of molecules that possess immunosuppressive activity, further facilitating tumor progression and metastasis. Elucidating the role chemokines and their receptors play in the formation and metastasis of tumors may aid in cancer management and treatment.

### Available Chemokine Products

- Recombinant Chemokines
- Human Chemokine Sampler Packs
- Polyclonal Antibodies
- Monoclonal Antibodies
- Labeled Antibodies
- ELISA Kits
- ELISA Development Reagents
- Flow Cytometry Kits
- cDNA Expression Arrays
- mRNA Quantitation Kits
- Primer Pairs

## Reference

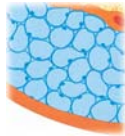
- Payne, A.S. & L.A. Cornelius (2002) *J. Invest. Dermatol.* **118**:915.

## ***R&D Systems has cancer research reagents applicable for...***

every level of biological organization



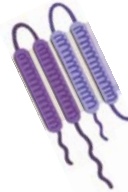
Organism



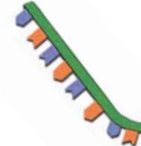
Tissue



Cell



Protein



RNA



DNA

a wide variety of experimental models

- Bovine
- Canine
- Chicken
- Cotton rat
- Feline
- Goat
- Human
- Mouse
- Porcine
- Rat
- Rhesus Macaque
- Viral
- Zebrafish

## ***R&D Systems & everything cytokine beyond***

- Recombinant and Natural Proteins
- Cell Differentiation & Expansion Kits
- Antibodies
- Signal Transduction Kits & Reagents
- ELISA Kits & Development Reagents
- Apoptosis Detection Kits & Reagents
- ELISpot Kits & Development Reagents
- DNA Damage & Repair Kits & Reagents
- mRNA Quantitation Kits
- cDNA Expression Arrays
- Cytokine Analytical Testing Service
- Basement Membrane Extracts
- Cell Enrichment Columns
- Primer Pairs

# www.RnDSystems.com

## NEW On-line Pricing

- Pricing is now available for US customers.
- International customers should refer to their distributor for accurate pricing.

## Searching:

- Entire site - search our entire database of scientific articles and technical information.
- Product search - search over 5500 products by product category, factor, species, catalog number or keyword.
- Unable to find a product that you are searching for? Submit a product request form.

## Live R&D Support

- Real time customer service and technical support are available.
- Reference database - information regarding the use of our products in published citations (e.g. specific applications, sample types, species cross-reactivity and other relevant information) is available upon request.

## Product Data Inserts

- Copies of all product inserts are available on-line.

## Reviews & Technical Notes

- Mini-reviews, troubleshooting guides, cytokine-related facts (e.g. WHO conversion table, abbreviations, accepted nomenclature), and recommended protocols for a variety of applications are available on-line.

## Cytokine Bulletin

- Quarterly scientific newsletter highlights the latest advances in cytokine biology.
- Access current and archived issues.

## Additional Literature

- Access archived issues of previous *In Scope* product newsletters.
- Product application guides.
- *De Novo* new product newsletters - browse past issues of our *De Novo* product newsletter for articles and product highlights.

## Weekly New Product E-mail

- Sign up to receive a weekly update on newly released products. Simply click on the Subscribe link at [www.RnDSystems.com](http://www.RnDSystems.com)

The screenshot displays the RnD Systems website interface. On the left, there is a navigation menu with links for 'site search', 'product search', 'login for U.S. pricing', 'mail us', 'LIVE R&D SUPPORT', 'catalog', 'new products', 'reviews & tech notes', 'cytokine bulletin', 'customer service', 'hematology', 'job opportunities', 'TECHNE', and 'home'. The main content area features a header with the RnD Systems logo and the tagline 'everything cytokine & beyond'. Below the header, there is a section titled 'We strive to develop and manufacture the highest quality and most extensive selection of cytokine-related reagents available in the research market today, while also providing superior customer and technical support.' This is followed by a list of 'Our products include:' such as Cytokine Multiplex Assays, Recombinant & Natural Proteins, Antibodies, ELISA/Assay Kits & Development Reagents, ELISPOT Kits & Reagents, mRNA Quantitation Kits, Cell Enrichment Columns, Cell Differentiation & Expansion Kits, Signal Transduction Kits & Reagents, Apoptosis Detection Kits & Reagents, DNA Damage & Repair Reagents, cDNA Expression Arrays, Basement Membrane Extracts, Primer Pairs, and Assay Services. A 'New Products for the week of March 2, 2003' section lists items like Human MCP-1/CCL23 Quantitative ELISA Kit, Human MMP-2 Quantitative ELISA Kit, Mouse TRGF-2 Biotinylated Affinity Purified Fab, Mouse TRGF-2 Biotinylated Affinity Purified Fab, and Mouse Angiogenesis-like 3 Biotinylated Affinity Purified Fab. There are also sections for 'Product Highlight' and 'Literature Available'.

## New for 2003... R&D Systems' aka CD-ROM

The **aka** CD-ROM facilitates access to R&D Systems' web-based cytokine nomenclature database. **aka** includes hundreds of proteins, their most recognized abbreviations, full names, systematic names (if applicable), aliases, and gene sequence links. Please visit [www.RnDSystems.com/aka09](http://www.RnDSystems.com/aka09) to request your copy today.

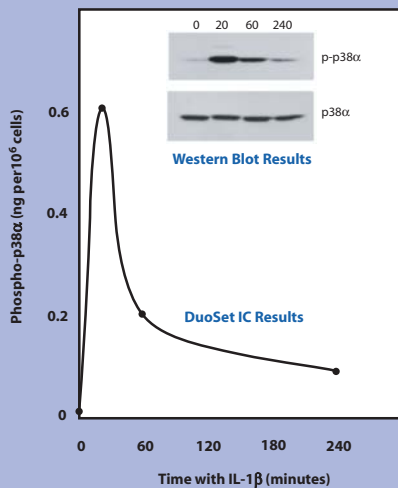


## DuoSet® IC ELISA Development Systems

DuoSet IC ELISA Development Systems have been developed for several important intracellular factors involved in apoptosis and signal transduction. They offer a fast, economical, and quantitative alternative to Western blot analysis that is more amenable to large-scale screening projects.

### ELISA vs. Western Blot

Measurement of phosphorylated p38 $\alpha$  in IL-1 $\beta$  (10 ng/mL) treated human HepG2 cells



### Available Kits

Analyte	Cat #
Bcl-2	DYC827
Bcl-x <sub>L</sub>	DYC894
Caspase-9 (Active)	DYC830
Cytochrome c	DYC897
Phospho-p38 $\alpha$ (T180/Y182)	DYC869
Total p38 $\alpha$	DYC8691
Phospho-p70 S6 Kinase (T389)	DYC896
Phospho-RSK1 (S380)	DYC892
SMAC/Diablo	DYC789
Survivin	DYC647

### Kit Contents\*

(Available in 2, 5, and 15 plate kit packs)

- Coating Antibody
- Standard
- Biotinylated Detection Antibody
- Streptavidin-HRP

\*The Caspase-9 assay is a novel and unique activity assay with different kit contents than those listed. See product insert for details.

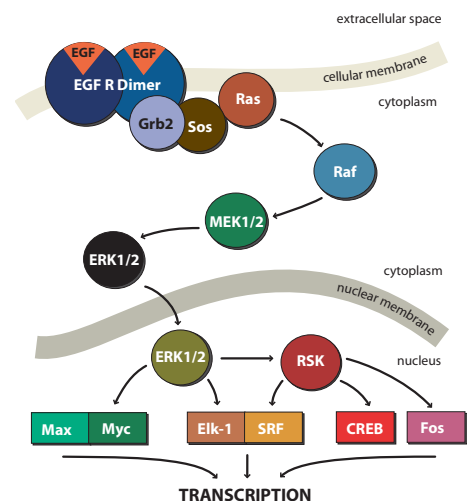
# ERK Activation and Growth Factor Signaling

Growth factor stimulation of intracellular signal transduction pathways is of considerable interest to the cancer researcher because alterations in these processes can lead to cellular transformation. The evolutionarily conserved mitogen-activated protein kinase (MAPK) signaling pathways, particularly the growth factor-activated pathway defined by extracellular signal-regulated kinase-1 (ERK1) and ERK2, are functionally linked to tumorigenesis by their roles in proliferation, differentiation and cell survival.<sup>1</sup> Initially isolated and cloned as kinases responsive to insulin and NGF stimulation, both ERKs are activated by dual threonine and tyrosine phosphorylation at T202/Y204 for ERK1 and T183/Y185 for ERK2.<sup>2,3</sup>

During growth factor stimulation, the ERK phosphorylation cascade is linked to cell surface receptor tyrosine kinases (RTKs) and other signaling proteins with known oncogenic potential. Overall, activating mutations within the ERK pathway are detected in nearly one-third of all cancers.<sup>1</sup> For example, ligand binding or transforming overexpression of the EGF receptor (EGF R) results in receptor dimerization and tyrosine autophosphorylation *in trans*. Phosphotyrosine 1068 of the activated EGF R is a direct binding site for the Grb2 adaptor molecule,<sup>4</sup> which recruits the guanine nucleotide exchange factor Sos.<sup>5</sup> Sos enhances GDP release and GTP binding to the membrane-bound product of the proto-oncogene *c-ras*. Ras-GTP binds the product of another proto-oncogene, *c-raf*, bringing this MAPK kinase kinase (MAP3K) to the plasma membrane where its activity is enhanced.<sup>6</sup> Phosphorylated Raf activates the dual specificity MAPK kinases MEK1 and MEK2,<sup>7</sup> which in turn phosphorylate ERK1 and ERK2.

Upon phosphorylation, nuclear translocation of ERK1 and ERK2 is required for growth

factor-induced gene expression and DNA replication.<sup>8</sup> In the nucleus, these ERKs phosphorylate a wide array of transcription factors. Among the best-characterized substrates are ternary complex factors (TCFs), including Elk-1, which are directly phosphorylated by ERK1 and ERK2 at multiple sites.<sup>9</sup> Upon complex formation with serum response factor (SRF), phosphorylated TCFs transcriptionally activate the numerous mitogen-inducible genes regulated by serum response elements.<sup>10</sup> Another direct target of these ERKs is the product of proto-oncogene *c-myc*, a short-lived phospho-protein involved in multiple aspects of growth control. Following phosphorylation at two sites within its transactivation domain, Myc activates transcription as a heterodimeric partner with Max.<sup>11</sup> ERK1 and ERK2 also regulate transcription indirectly by phosphorylating the 90 kDa ribosomal protein S6 kinases (RSKs). RSKs activate the immediate early gene transcription factor CREB<sup>12</sup> and the product of proto-oncogene *c-fos*, an AP-1 component.<sup>13</sup>



**Figure 1.** The prototypical MAPK signaling cascade initiated by a growth factor binding to its receptor thus resulting in the activation of ERK1/2 nuclear signaling is represented.

### References

1. Seger, R. & E. Krebs (1995) *FASEB J.* **9**:726.
2. Boulton, T. *et al.* (1990) *Science* **249**:64.
3. Boulton, T. *et al.* (1991) *Cell* **65**:663.
4. Rojas, M. *et al.* (1996) *J. Biol. Chem.* **271**:27456.
5. Li, N. *et al.* (1993) *Nature* **363**:85.
6. Jelinek, T. *et al.* (1996) *Mol. Cell. Biol.* **16**:1027.
7. Kyriakis, J. *et al.* (1992) *Nature* **358**:417.
8. Brunet, A. *et al.* (1999) *EMBO J.* **18**:664.
9. Marais, R. *et al.* (1993) *Cell* **73**:381.
10. Gille, H. *et al.* (1995) *EMBO J.* **14**:951.
11. Gupta, S. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**:3216.
12. Xing, J. *et al.* (1996) *Science* **273**:959.
13. Chen, R-H. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90**:10952.

# Human Tissue Kallikreins as Cancer Markers

The ability to detect cancer at an early stage can make a dramatic difference in clinical outcome. Furthermore, the ability to distinguish more aggressive from less aggressive forms can have a significant bearing on the selection of treatment options. Although the development of microarray technology shows great promise in advancing diagnostic and therapeutic goals,<sup>1-3</sup> at the present time, individual biomarkers, along with more traditional measures such as tumor size, location, TNM (tumor, node, metastasis) stage grouping, DNA ploidy, and histologic parameters have been more frequently utilized. One such well-studied biomarker is prostate-specific antigen (PSA).<sup>4</sup> PSA is a member of the human tissue kallikrein family.<sup>4</sup>

Plasma and human tissue kallikreins are serine proteases that exhibit significant structural and functional differences.<sup>4</sup> The human plasma kallikrein is encoded by a single gene on chromosome 4q35 and is expressed exclusively by liver cells. In contrast, the human tissue kallikrein family consists of at least 15 members, whose genes have been mapped to an ~300 kb region on chromosome 19q13.4.<sup>4-6</sup> All of the human tissue kallikreins are serine proteases that contain a conserved catalytic triad composed of His, Asp, and Ser residues.<sup>4</sup> The genes

contain 5 coding exons of similar or identical size and intron phases that are conserved throughout the family.<sup>4</sup> At both the nucleotide and amino acid level, the human tissue kallikreins share 40 to 80% identity. The greatest degree of similarity is localized to the catalytic triad region and 10 to 12 highly conserved cysteine residues.<sup>4</sup> It appears that steroid hormones act as transcriptional regulators of several family members.<sup>4-7</sup> Human tissue kallikreins were initially found in prostate and breast tissue, although more recent RT-PCR analysis has revealed diverse expression patterns in a wide array of tissue types.<sup>4-7</sup>

Many human tissue kallikrein family members have proven or potential value as markers for breast, ovarian, prostate or testicular cancer (see Table).<sup>5,6,8</sup> Both serum protein and mRNA levels can have predictive value.<sup>5,6,9</sup> Physiological roles for human tissue kallikreins have not been fully elucidated. However, reported signaling pathways involved in tumorigenesis suggest that in addition to their use as markers, they may provide attractive therapeutic targets.<sup>5-7</sup> It is predicted that the availability of human tissue kallikrein proteins will facilitate studies that may yield valuable information on drug design.<sup>10,11</sup>

MEMBER	ALTERNATE NAMES	CANCER TYPES
KLK1	Pancreatic/renal kallikrein (hPRK)	
KLK2	Human glandular kallikrein 1 (HGK-1)	Prostate, Breast
KLK3	Prostate-specific antigen (PSA)	Breast, Prostate
KLK4	Kallikrein-like 1 (KLK-L1) protein; Prostate; Enamel Matrix Serine Protease 1 (EMSP1)	Ovarian, Breast, Prostate
KLK5	Human Stratum Corneum Tryptic Enzyme (HSCTE); Kallikrein-like 2 (KLK-L2) protein	Breast, Ovarian, Prostate, Testicular
KLK6	Protease M; Neurosin; Zyme; Myelencephalon-specific Protease	Breast, Ovarian
KLK7	Human Stratum Corneum Chymotryptic Enzyme (HSCCE)	Ovarian
KLK8	Tumor-associated Differentially Expressed Gene-14 (TADG-14); Neuropsin; Ovasin; Brain Serine Protease 1 (BSP1)	Ovarian
KLK9	Kallikrein-like 3 (KLK-L3) protein	Ovarian
KLK10	Normal epithelial cell-specific 1 (NES1) protein	Breast, Ovarian, Testicular
KLK11	Trypsin-like Serine Protease (TLSP); hippostasin	Ovarian, Prostate
KLK12	Kallikrein-like 5 (KLK-L5) protein	Breast
KLK13	Kallikrein-like 4 (KLK-L4) protein	Breast
KLK14	Kallikrein-like 6 (KLK-L6) protein	Breast, Ovarian, Testicular
KLK15		Breast, Ovarian, Prostate

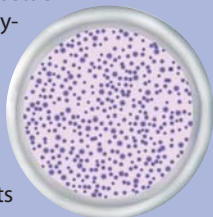
## References

- van de Vijver, M.J. *et al.* (2002) *N. Engl. J. Med.* **347**:1999.
- Kallioniemi, A. (2002) *N. Engl. J. Med.* **347**:2067.
- Sauter, G. & R. Simon (2002) *N. Engl. J. Med.* **347**:1995.
- Yousef, G.M. & E.P. Diamandis (2001) *Endocrine Rev.* **22**:184.
- Diamandis, E.P. & G.M. Yousef (2002) *Clin. Chem.* **48**:1198.
- Yousef, G.M. & E.P. Diamandis (2002) *Tumour Biol.* **23**:185.
- Yousef, G.M. & E.P. Diamandis (2002) *J. Biol. Chem.* **277**:1045.
- Diamandis, E.P. *et al.* (2001) *Trends Endocrinol. Metab.* **11**:54.
- Diamandis, E.P. *et al.* (2002) *Cancer Res.* **62**:295.
- Bernett, M.J. *et al.* (2002) *J. Biol. Chem.* **277**:24562.
- Gomis-Rüth, F.X. *et al.* (2002) *J. Biol. Chem.* **277**:27273

## Cytokine ELISpot Assays

*The benchmark for monitoring the quality and magnitude of antigen-specific immune responses.*

Cytokine ELISpot assays measure the relative number of cells secreting a specific cytokine. Cells are allowed to settle and incubate on an antibody-coated membrane in a 96-well microplate. Secreted analyte is captured locally (near the cell) by the immobilized antibodies. Addition of detection reagents results in the formation of blue spots on the membrane where each cell has settled and secreted cytokine. The spots can be counted automatically with an ELISpot plate reader or manually with a stereomicroscope.



Individual cytokine-secreting cells can be detected at frequencies well below 1 in 100,000, so *in vitro* cell expansion is not required before running an ELISpot assay. Unlike cytotoxicity assays, ELISpot results are highly reliable and reproducible.

The quality of the immune response (Th1- or Th2-dominated) is determined by the cytokine being secreted, and the magnitude (clonal size) is determined by the number of cells secreting that cytokine.

## Complete Kits

Kit	Species	Kit	Species
GM-CSF	● ■	IL-6	● ◆
IFN-γ	● ◆ ● ◆ ☆ ■	IL-8	●
IL-1β	●	IL-10	◆ ● ■
IL-2	● ● ◆	IL-13	◆
IL-4	● ◆	Latent TGF-β1	●
IL-5	●	TNF-α	● ◆

● Human      ◆ Rat      ☆ Canine  
 ■ Mouse      ● Feline      ★ Primate

## Reagents

ELISpot Development Modules are also available. Visit [www.RnDSystems.com](http://www.RnDSystems.com) for details.

## ELISA/ELISpot WORKSHOP

*For those less familiar with ELISpot assays, R&D Systems offers a combined ELISA/ELISpot workshop at least twice each year. Please visit [www.RnDSystems.com](http://www.RnDSystems.com) for details.*

**Quantikine® ELISAs**

- Colorimetric sandwich ELISAs for human mouse, rat, and porcine cytokines, chemokines, growth factors, adhesion molecules, MMPs, caspases, NOS, and related molecules.

**Quantikine® HS ELISAs**

- Highly sensitive colorimetric ELISAs for the quantitation of human cytokines. These kits employ an alkaline phosphatase-based color amplification system that is read at 490 nm and can detect femtogram levels of analyte.

**QuantiGlo® ELISAs**

- Chemiluminescent ELISAs for human cytokines. Increased sensitivity and a wider dynamic range reduce the need for extra dilutions. A chemiluminescent plate reader is required to run these kits.

**Quantikine® IVD®**

- Colorimetric sandwich ELISAs for in vitro diagnostic use. Kits for Epo, sTfR, and  $\beta_2M$  are currently available.

**Quantikine® mRNA ELISAs**

- Colorimetric, microplate-based assays for the measurement of cytokine-specific mRNA transcripts. No gels or radioactive reagents are required.

**Fluorokine® MAP**

- Coated-bead assays for the simultaneous measurement of multiple cytokines in the same sample on the Luminex® platform.

**Fluorokine® E**

- Captured-enzyme activity assays for the quantification of active and total MMPs. A fluorometric plate reader is required to run these kits.

**ELISpot**

- Highly sensitive, microplate-based assays for the detection of absolute numbers and frequencies of cytokine secreting cells.

**Other Assays**

- Colorimetric assays for the detection of eicosanoids, hormones, cyclic nucleotides, nitric oxide, and related factors.

**Assay Services**

- R&D Systems' Analytical Testing Service can test your samples using any of our more than 200 assays. For more information, please call or e-mail us at [AssayServices@RnDSystems.com](mailto:AssayServices@RnDSystems.com).

R&D Systems'  
**Quantikine**  
ELISAs  
Precision Cytokine Measurement

**The most  
referenced name in  
complete, sample-  
validated cytokine  
assays** [www.RnDSystems.com](http://www.RnDSystems.com)

**R&D**  
SYSTEMS

**R&D Systems**

614 McKinley Place N.E.  
Minneapolis, MN 55413  
Fax: (612) 656-4400  
Tel: (612) 379-2956  
1-800-343-7475

Change Service Requested



Printed on recycled  
paper 10% post  
consumer waste.

PRSR STD  
U.S. POSTAGE  
PAID  
R&D SYSTEMS