


degenerative disease of the retina, which is caused by progressive rod photoreceptor death. The autosomal dominant form of retinitis pigmentosa is often caused by missense or nonsense mutations in the rod opsin gene that is then translated into nonfunctional and toxic protein. Treating this kind of disease is somewhat different than treating a “simple” protein deficiency disease, because the reconstitution of a functional protein is not enough. One needs to rid the eye of the mutant and harmful protein as well. Therefore, Hauswirth’s group uses ribozymes, RNA enzymes that can cleave a target RNA with high specificity. In their approach (6), the adeno-associated virus codes for a ribozyme that is able to distinguish between the normal and the mutant rod opsin mRNA, which is then cleaved and is not able to be translated (Figure 2). The virus is injected under the retina and the ribozyme is transcribed under the control of the rod opsin promoter, which increases the specificity of the treatment. So far the results are quite good: in one pig and several rat models, a single injection has restored up to 80% of the rod photoreceptors compared to the

contralateral (control) eye. The effect has not decreased in over two years, which has been explained by a small amount of viral DNA incorporation to the recipient cells. This AAV-ribozyme-based strategy holds great promise for the therapy of retinitis pigmentosa, as well as other dominantly inherited diseases.

Summary

In summary, the investigators that spoke at the Promega Neurosciences symposium were impressive, and the results of their work spoke for themselves. The enjoyable and educational day was closed with a view on the future of new strategies and technologies by the keynote address lecturer, **Richard C. Mulligan**. His talk abolished the last doubts, if any, of the possibilities of gene therapy for neurodegenerative diseases. 

References


1. Keirstead, H.S. *et al.* (1999) *J. Neurosci.* **19**, 7529.
2. Brustle, O. *et al.* (1998) *Nat. Biotech.* **16**, 1040.
3. Flax, J.D. *et al.* (1998) *Nat. Biotech.* **16**, 1033.
4. Johansson, C.B. *et al.* (1999) *Cell* **96**, 25.
5. Brooks, A.I. *et al.* (1997) *Nat. Biotech.* **15**, 57.
6. Lewin, A.S. *et al.* (1998) *Nat. Med.* **4**, 1033.

Anti-GFAP pAb

Identify glial cells in mixed neuronal cell population

Promega’s Anti-GFAP (glial fibrillary acidic protein) pAb is raised in rabbits against bovine glial fibrillary acidic protein (GFAP), a specific marker of astrocytes in the CNS, and is qualified for immunostaining applications.

Applications (recommended dilutions)

- **Immunocytochemistry:** 1:1,000.
- **Immunohistochemistry:** 1:1,000.
- **Western Blotting:** 1:1,000. 

Astrocyte Cell Marker

Product	Size	Cat. #
Anti-GFAP pAb	100µg	G5601

Features of the Anti-GFAP pAb.

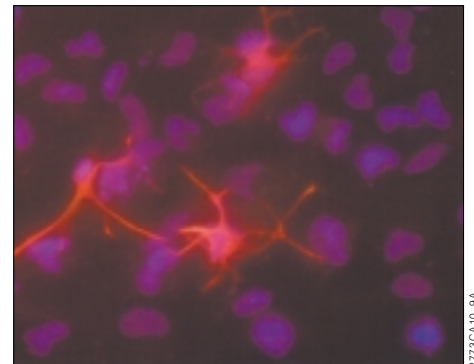
Specificity: Human, bovine and rat GFAP; not recommended for mouse.

Form: Purified rabbit IgG; supplied at 1mg/ml in PBS containing 50µg/ml gentamicin.

Immunogen: Purified GFAP from bovine spinal cord.

Staining of astrocytes by Anti-GFAP pAb. ▶

Anti-GFAP pAb in conjunction with anti-Cy[™]3 conjugate labels astrocytes (*red*) in mixed population of rat neural progenitor cells. Nuclei are stained with DAPI (*purple*). Protocols developed and performed at Promega.



CaspACE™ FITC-VAD-FMK In Situ Marker

Cell-permeable marker for caspase activity

CaspACE™ FITC-VAD-FMK In Situ Marker is a FITC (fluorescein isothiocyanate) conjugate of the irreversible caspase inhibitor, VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone). This compound is cell permeable allowing for delivery of the marker into the cell where it irreversibly binds activated caspases and the FITC label allows for a single reagent addition to directly detect caspase activity in situ. CaspACE™ FITC-VAD-FMK is supplied as a 5mM solution in DMSO and is intended for in situ monitoring of caspase activity.

For additional information on Promega's line of reagents for the study of apoptosis, please see our web site at:

www.promega.com/cellsig/ 

Benefits of the CaspACE™ FITC-VAD-FMK In Situ Marker.

In Situ Marker: Detect apoptosis in situ.

Fast: Results in 30 minutes.

Easy: Add marker, incubate, wash and view fluorescence.

M-MLV RT, RNase H Minus ('Deletion' and 'Point Mutant' forms)

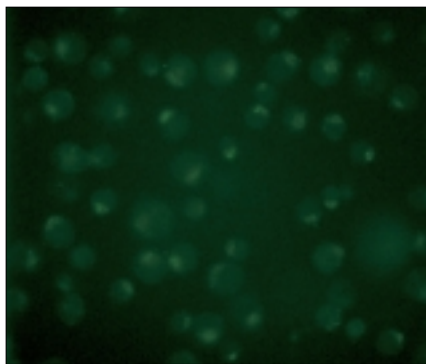
Now available worldwide from Promega

Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus (M-MLV RT H-) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5kb). This is a form of M-MLV Reverse Transcriptase that has been genetically altered to remove the associated RNase H activity. Although many researchers are successful in using M-MLV RT, RNase H+,

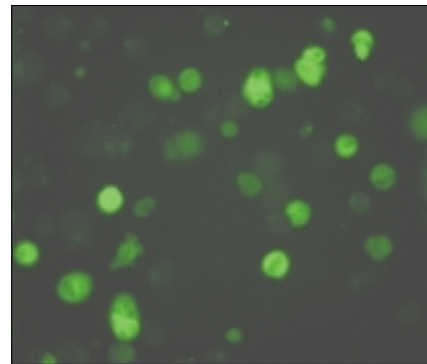
Apoptosis Detection

Product	Conc.	Size	Cat.#
CaspACE™ FITC-VAD-FMK In Situ Marker	5mM	50µl	G7461
	5mM	125µl	G7462
Caspase Inhibitor Z-VAD-FMK	20mM	50µl	G7231
	20mM	125µl	G7232

A.



B.




▲ CaspACE™ FITC-VAD-FMK In Situ Marker labels Jurkat cells undergoing apoptosis.

Panel A: Untreated Jurkat cells. Panel B: Anti-Fas mAb (PanVera)-treated Jurkat cells. CaspACE™ FITC-VAD-FMK In Situ Marker was used at a final concentration of 10µM.

Caspase Inhibitor Z-VAD-FMK

Caspase Inhibitor Z-VAD-FMK (valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone) an irreversible, pan-caspase inhibitor, is provided at 20mM in DMSO for convenient

addition to cell culture or extracts. The suggested concentration for use in the anti-Fas mAb-treated Jurkat cell culture model system is 20µM. 

Modifying Enzymes

Product	Size	Cat.#
M-MLV RT, RNase H-, Deletion Mutant	10,000 units	M5301
M-MLV RT, RNase H-, Point Mutant	10,000 units	M3682
	50,000 units	M3683

for analytical and some preparative cDNA applications, reverse transcriptases lacking RNase H activity provide another option for the preparation of long cDNAs and libraries containing a high percentage of full-length cDNA. The 'Point Mutant' enzyme possesses increased thermostability, thus preventing problems associated with RNA secondary structure.

Applications

- First strand synthesis of cDNA from RNA molecules.
- cDNA library construction.
- RT-PCR.

For additional information on this and other modifying enzymes available from Promega, please see our web site at:

www.promega.com/ena/ 

SignaTECT® CaM KII Protein Kinase Assay System

Sensitive method to quantitate CaM KII in crude cell extracts

The SignaTECT® Calcium/Calmodulin-Dependent (CaM KII) Protein Kinase Assay System^(a) provides an improved method by which to quantitate CaM KII protein kinase activity, both in purified enzyme preparations and in cell or tissue extracts.

The system overcomes the problem of nonspecific substrate binding by using a biotinylated peptide substrate that is selective for CaM KII in conjunction with Promega's SAM²® Biotin Capture Membrane. The high binding capacity of the SAM²® Membrane for the CaM KII biotinylated substrate and the low backgrounds observed with this system maximize the signal-to-noise ratio. This assay system has been successfully used with purified CaM KII enzyme and crude rat brain extract.

InCELLect™ Peptides

Cell-permeable anchoring inhibitory peptides

InCELLect™ Peptides are cell-permeable anchoring inhibitory peptides for studying cAMP-dependent protein kinase-mediated signaling events. InCELLect™ AKAP St-Ht31 Inhibitor Peptide is a stearylated (St) form of the peptide Ht-31 derived from the human thyroid AKAP, A-Kinase-Anchoring Protein. This peptide has been shown to bind to the RII subunit of PKA and disrupt RII interaction with AKAP. As a result of this disruption, PKA is delocalized from the intended physiological cellular targets. The presence of the hydrophobic stearylated moiety enhances the cellular uptake of the peptides, allowing them to be used to study

^(a)Patent Pending.

Signal Transduction


Product	Size	Cat.#
SignaTECT® CaM KII Protein Kinase Assay System	96 reactions	V8161

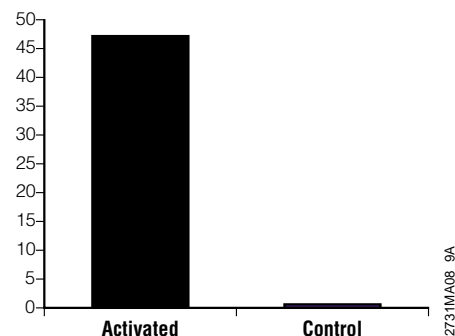
Features and Benefits of the SignaTECT® CaM KII Protein Kinase Assay System.

Specificity: CaM KII-specific biotinylated substrate for high specificity.

Robustness: Streptavidin capture of substrate results in high signal-to-noise ratio.

Easy: Designed for sensitive quantitation of CaM KII in cell and tissue extracts.

All SignaTECT® Assay Systems^(a) use the SAM²® Biotin Capture Membrane, produced by a proprietary process that generates a high density of streptavidin on the membrane matrix. The streptavidin matrix provides rapid, quantitative capture of biotinylated substrate molecules based on the strong affinity of biotin for streptavidin ($K_d=10^{-15}$). The SAM²® Membrane can bind biotinylated substrate at a minimum of 1.3nmol/cm². In addition, the membrane has been optimized for low nonspecific binding. 



▲ Detection of CaM KII activity in rat brain.

Extract was isolated from rat brain, and CaM KII activity was measured using the SignaTECT® CaM KII Protein Kinase Assay System.

Signal Transduction

Product	Size	Cat.#
InCELLect™ AKAP St-Ht31 Inhibitor Peptide	150µl	V8211
InCELLect™ St-Ht31P Control Peptide	150µl	V8221

Features of the InCELLect™ Cell-Permeable Peptides.

Real-Time Studies: Study real-time physiological effects related to PKA signaling.

Permeability: Stearylated group makes peptides cell permeable, eliminating the need for microinjection.

Selectivity: Selective and specific peptide sequence that binds to the RII subunit of PKA and disrupts PKA anchoring.

Control: Negative control peptide (Cat.# V8221) does not bind RII subunit of PKA.

PKA signaling in intact cells in culture. The InCELLect™ St-Ht31P Control Peptide^(a) varies in two amino acids and does not disrupt RII-AKAP binding. This peptide has been used as a negative control peptide for the study of InCELLect™ AKAP St-Ht31 Inhibitor Peptide. The peptides are supplied in a 10mM stock solution for addition to cell

culture media. Optimal concentration and incubation times must be determined for each experimental system.

For more product information, please visit our web site at: www.promega.com/cellsig/ or request Application Note #AN074. 