

Proteins

The range of proteins R&D Systems provides includes:

- cytokines
- growth factors
- chemokines
- receptors
- proteases
- adhesion molecules

This range has been developed for several different species to facilitate many experimental procedures. All proteins are produced using quality assured procedures with stringent production controls to give you confidence in the product you have chosen.



“I have found R&D Systems’ recombinant cytokines to be extremely consistent from lot to lot. With R&D Systems’ cytokines, we have obtained very reproducible and consistent results.”

Dr. Douglas D. Bannerman
Division of Hematology,
University of Washington,
Seattle, WA, USA

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Protein Folding

The production of high quality, biologically active, recombinant proteins depends on the folding and assembly of those proteins into their native structures so that they may function correctly. Fortunately, the entire plan for protein folding into three-dimensional structures, assembly of those structures into complexes, and therefore biological activities is genetically encoded.¹ *In vivo*, as nucleotide sequences are translated into amino acid sequences, the growing polypeptide chain folds into its secondary and tertiary structures at the same time.^{1,2} The sequence of types of amino acids and how they interact with their solvent environment combine to cause the polypeptide chain to fold and compact into the structure that is most thermodynamically favorable.¹ Although protein folding is a spontaneous and autonomous process, it is not without molecular helpers *in vivo*. To increase efficiency, via speed and prevention of misfolding and aggregation, proteins called foldases and chaperones often participate in the folding process.^{1,2}

It is sometimes not feasible to produce accurately-folded, soluble, recombinant proteins *in vivo*. In these cases, aggregated proteins are refolded *in vitro*. Aside from the absence of foldases and chaperones, and the possibility of slight mechanistic variability, many studies have shown that *in vivo* and *in vitro* protein folding and assembly can produce exactly the same final product.¹ There are three major parameters that must be evaluated and controlled to achieve successful protein folding and assembly *in vitro*: temperature, protein concentration, and solvent conditions. Optimally, refolding should be performed at a low temperature and a low concentration to prevent the formation of aggregates. Low temperature tends to reduce the influence of hydrophobic interactions, one of the main culprits of aggregation. Low concentration discourages the specific folding kinetics associated with pathways that lead to misfolding and aggregation. Appropriate solvent conditions are also chosen to maximize correct folding and minimize misfolding and aggregation. The dynamic *in vivo* solvent environment cannot be duplicated in the laboratory. However, neutral pH and several refolding buffer additives aid in mimicking some qualities of the cytoplasm. For example, carbohydrates are added to increase viscosity, oxidizers are added to aid disulfide bond formation, and detergents are added to prevent aggregation.^{1,3}

There are innumerable examples in which misfolding alters or obliterates the functional capabilities of a protein, dramatically impacting not just the particular cell type that expresses it, but also the organism as a whole. Cystic fibrosis, sickle cell anemia, and phenylketonuria, are all genetic disorders caused by

mutations in single genes encoding proteins that are, as a result, misfolded or misassembled, and thus have defective activities.⁴ These are only a few examples, but are clear testimonials to the necessity of accurate protein folding and assembly in order to ensure correct biological function.

R&D Systems employs several different expression systems for the high throughput production of recombinant proteins. Large proteins, which require some form of post-translational modification, are generally produced in a cell culture format. In these cells, polypeptide chains fold spontaneously, possibly are modified, potentially assemble into oligomers, and are secreted into the cell culture supernatant from which they are later purified. In this scheme, there is no need for the scientist to fold or refold the protein, as the cellular environment encourages correct, autonomous, protein folding and assembly *in vivo*. Smaller, simpler proteins are generally produced in *E. coli*. Whereas, nascent polypeptide chains spontaneously and autonomously fold into their three-dimensional structures in *E. coli* as well, most proteins are deposited in inclusion bodies. This sequestration, while advantageous in its ability to protect against degradation, causes the proteins to lose their native conformations.^{2,3} In this scheme, the scientist must refold the protein *in vitro* after retrieval from the inclusion bodies and before purification.

At R&D Systems, protein purification and folding is carefully monitored to ensure that the structure of the proteins we provide is correct. The purification procedures employed treat *in vivo*-folded proteins as gently as possible to prevent precipitation and aggregation. Proteins that require *in vitro* refolding are folded slowly and carefully under critically evaluated conditions. Meanwhile, the generation of misfolded and aggregated byproducts is monitored and restricted to a very low level. The biological activity of every lot of protein is scrutinized in a bioassay, if available, to test its functional capabilities. We take great care and pride in the production of our protein products so that you receive structurally and functionally superior proteins to use in your experiments.

References

1. Jaenicke, R. and H. Lillie (2000) *Adv. Prot. Chem.* **53**:329.
2. Thomas, J.G. *et al.* (1997) *Appl. Biochem. Biotechnol.* **66**:197.
3. De Bernardez Clark, E. (2001) *Curr. Opin. Biotechnol.* **12**:202.
4. National Center for Biotechnology Information Web Site: www.ncbi.nlm.nih.gov/disease

Features and Benefits

Quality Control Each new lot of protein is tested side-by-side with previous lots for biological potency, endotoxin level and purity. This ensures that there is minimal lot-to-lot variability and requires minimal adjustments be made to established experimental protocols.

Specific Activity The specific activity of a protein is determined by bioassay or functional ELISA. Using an appropriate biological system, the protein is tested to ensure biological activity is consistent with literature-based expectations. The mass of protein required to elicit a 50% maximum response in the bioassay is called the ED₅₀. The ED₅₀ is stated on individual product datasheets. Each new lot of protein is required to give an ED₅₀ which falls within an expected range to ensure minimum lot-to-lot variation in potency. Each ED₅₀ quoted should be used as a guide only, researchers should define their own dose response ranges under their particular experimental conditions.

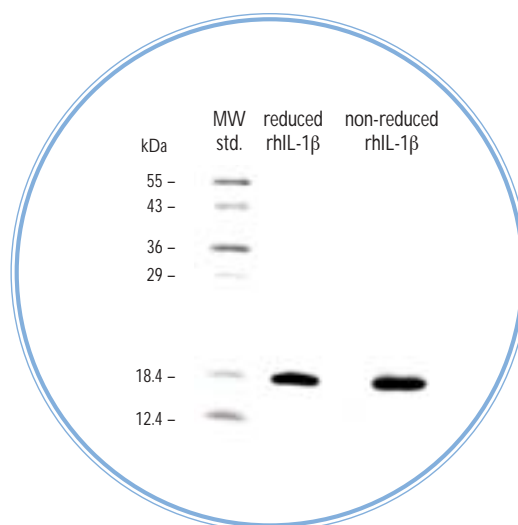
Endotoxin Level Each new production lot of protein is assessed for endotoxin using the Limulus amoebocyte lysate (LAL) assay. Low endotoxin levels are essential when proteins are used in biological systems that are sensitive to its effect. QC testing of endotoxin ensures that the biological response observed is due to specific action of the protein and not contaminants.

Formulation Most recombinant proteins are lyophilized from a sterile filtered solution containing a carrier protein, usually bovine serum albumin (BSA). This gives the lyophilized protein greater stability allowing easy handling and storage and maximizing recovery upon reconstitution. For applications where carrier protein is undesirable, e.g. *in vivo* experiments, carrier-free proteins are available.

Wide Range Available Due to limited sequence identity between different species, R&D Systems produces human, mouse, rat, cotton rat, porcine, canine, feline, rhesus macaque, *Drosophila*, zebrafish, and viral proteins.

Bulk Quantities When experimental protocols require large quantities of a particular protein, special bulk pack sizes can be purchased. This will reduce the cost per unit mass. Please contact us for a bulk order quote.

Purity Recombinant proteins are over 95% pure. Purity is assessed by silver staining an SDS polyacrylamide gel containing the electrophoresed protein. Due to the detection limit associated with silver staining, it is not possible to determine 100% purity. High purity ensures that the experimental outcome is a result of the protein of interest and not due to the presence of a contaminant.



Recombinant human IL-1β was run reduced and non-reduced on an SDS-PAGE gel, and silver stained.

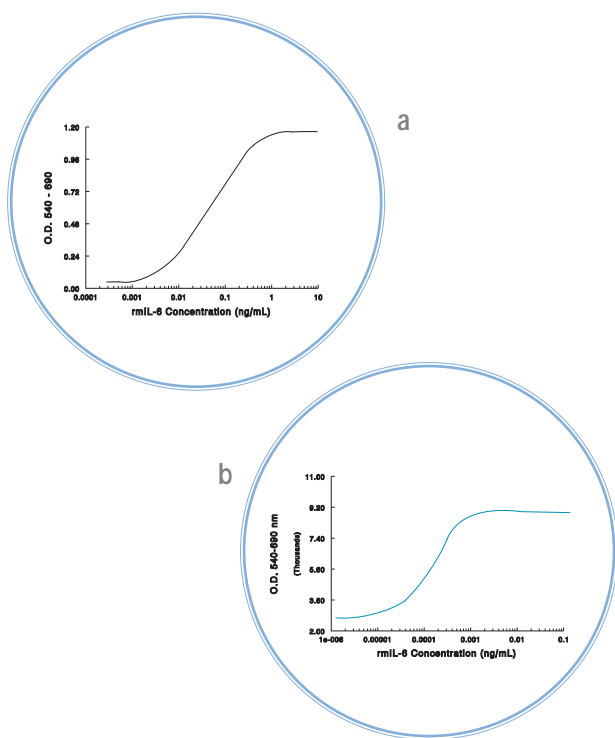
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Specific Activity

At R&D Systems, recombinant proteins are sold in units of mass per vial (μg or mg) with a specific activity range rather than as units of activity per vial.

How is Activity Measured?

The biological activity of a recombinant protein is routinely measured using a bioassay, e.g. chemotaxis or cell proliferation, or a functional ELISA. Due to a protein's ability to exert multiple biological activities, several acceptable bioassays may exist for a particular protein and several different cell types may respond. Different cell types have different sensitivities to a particular protein. Therefore, the activity of a protein is related to the cell type, as well as the bioassay employed. The graphs below demonstrate that different concentrations of recombinant mouse IL-6 are needed to induce proliferation of two different cell types. In these two commonly used mouse IL-6 assays, the B9 hybridoma cell line is approximately 100-fold more sensitive than the T1165.85.2.1 plasmacytoma cell line. Consequently, the same mass of IL-6 can be assigned two different units of activity.



a Mouse IL-6 stimulates the proliferation of the IL-6-dependent mouse plasmacytoma cells, T1165.85.2.1 in a dose-dependent manner. The ED_{50} for this effect is typically 0.02 ng/mL .

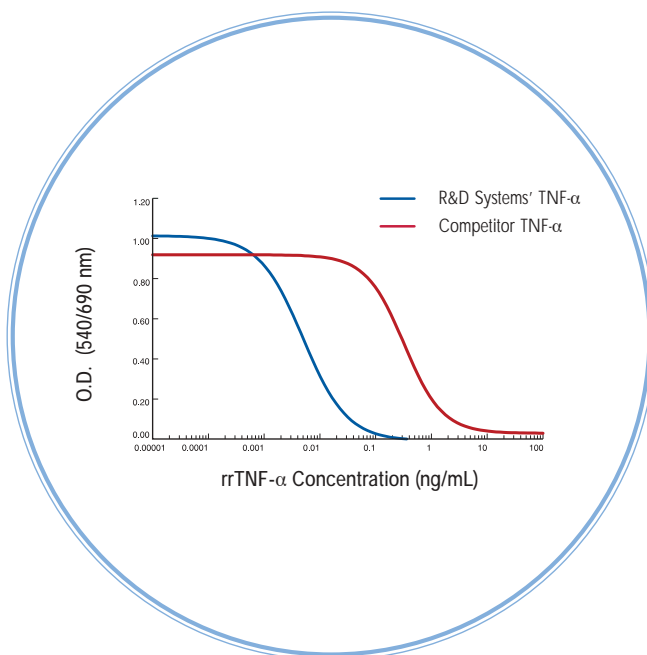
b Mouse IL-6 stimulates the proliferation of the IL-6-dependent B9 cells in a dose-dependent manner. The ED_{50} effect is typically 0.00026 ng/mL .

Each new lot of protein and each new set of experimental conditions should be tested in a titration (dose response) experiment. This needs to be done when a new experiment is set up or with a new lot or a new source of protein.

Can Proteins be Compared by Units?

A valid comparison between the bioactivity units of two proteins requires that the values be generated using the same bioassay. To follow a published protocol given in units, or to compare to another vendor's product sold in units, customers must examine how the author/vendor defined the unit. If the author/vendor compared their unit to the World Health Organization (WHO) standard unit, the information contained in the WHO Conversion Table found in R&D Systems' Catalog may prove useful as a starting point.

We strongly encourage customers comparing different vendors' proteins, especially when based on units of activity, to perform side-by-side titration experiments. For example, as shown in the figure below, results from a recent comparison of R&D Systems' recombinant rat TNF- α (rrTNF- α) and a competitor's rrTNF- α showed R&D Systems' protein to be 60-fold more active in the same bioassay. The biological activity was measured in a cytotoxic assay using a TNF- α susceptible mouse L-929 cell line in the presence of the metabolic inhibitor, actinomycin D. R&D Systems' rrTNF- α had an ED_{50} of 0.005 ng/mL while the competitor's rrTNF- α had an ED_{50} of 0.324 ng/mL .



Why is the ED₅₀ Provided as a Range?

Bioassays are a measure of biological response to a protein, therefore, any variation in a bioassay may also lead to variable results. Such variations could include cell density, age of the cells, passage number, nutritional state, culture medium and supplements used, as well as the technique of the assay operator. Due to the variable nature of bioassays, the same protein preparation may not give the same ED₅₀ on different occasions. The ED₅₀ range given on R&D Systems' protein inserts is typical for the preparation. All lots of the protein must demonstrate an ED₅₀ within that particular range in order to pass the quality control (QC) standards set by R&D Systems. All newly bottled lots of protein are compared in the same bioassay with a lot of the same protein that previously passed our QC standards.

How is Enzymatic Activity Measured?

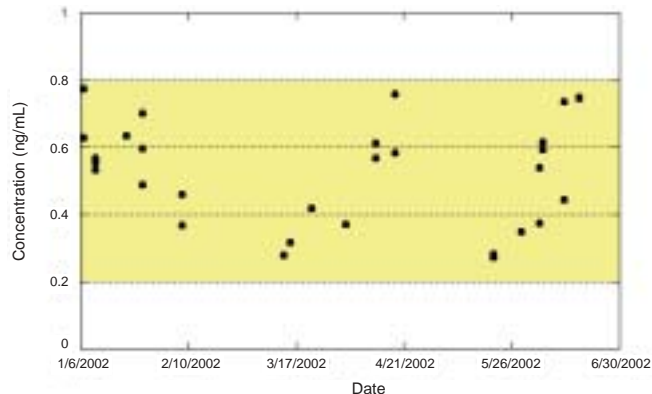
When a recombinant protein is an enzyme, specific activity is derived from an enzymatic assay. Each enzyme is tested for potency by cleavage of a substrate, and specific activity is expressed as pmoles/min/μg.

Are Bioactivity Units and International Units the same?

There are several sources of reference standard preparations for proteins. The most common is the WHO standard from National Institute for Biological Standards and Controls (NIBSC). Reference proteins are available for a limited number of proteins. When a standard

is available, R&D Systems compares the standard with the recombinant protein in the same bioassay. The result of this comparison is published in the catalog and the International Unit (IU) equivalent conversion is also provided.

The conversion between units and ED₅₀ values is not absolute as International Units are not derived solely from bioassay activity and ED₅₀ values can vary with slight changes in conditions. The best means of ensuring the correct dosage is to compare the new lot with the old lot, in a side-by-side dose response assay in the investigator's test system.

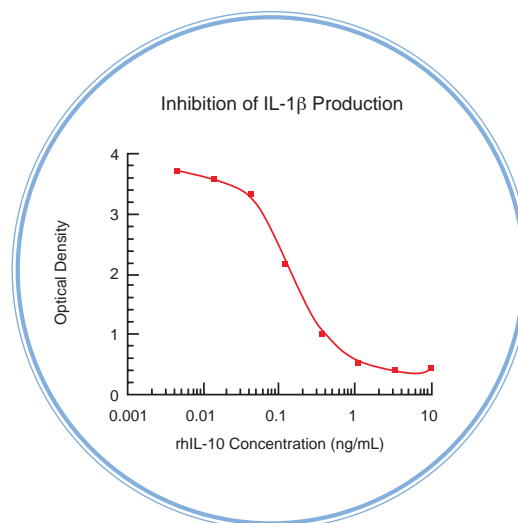


Recombinant human IL-6 was bioassayed over six months and showed ED₅₀ values within the range 0.2 – 0.8 ng/mL.

Applications – Cell Culture Studies

The physical and pathological role a recombinant protein plays is often measured in cell culture. As mentioned previously, it is essential that the protein is functionally active and free of contaminants for the response to be informative. Numerous bioassay protocols are available from R&D Systems' Technical Service Department and several protocols are available on our web site.

Human IL-10 inhibits IL-1β secretion by LPS-activated human peripheral blood mononuclear cells in a dose-dependent manner (Ralph, P. *et al.*, 1991, *J. Immunol.* **148**:808). The ED₅₀ for this effect is typically 0.1–0.25 ng/mL.



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Applications – In Vivo

Several animal models of human diseases are available to assist in discovering the specific roles a protein plays in that disease. Species cross-reactivity of many proteins allows elucidation of biological information from *in vivo* animal experiments even when using a non-species specific protein.

It is important to use carrier-free protein for injection so that the biological response invoked is a result of the protein of interest and not the carrier protein. Bulk quantities of protein are also available for *in vivo* animal research experiments.

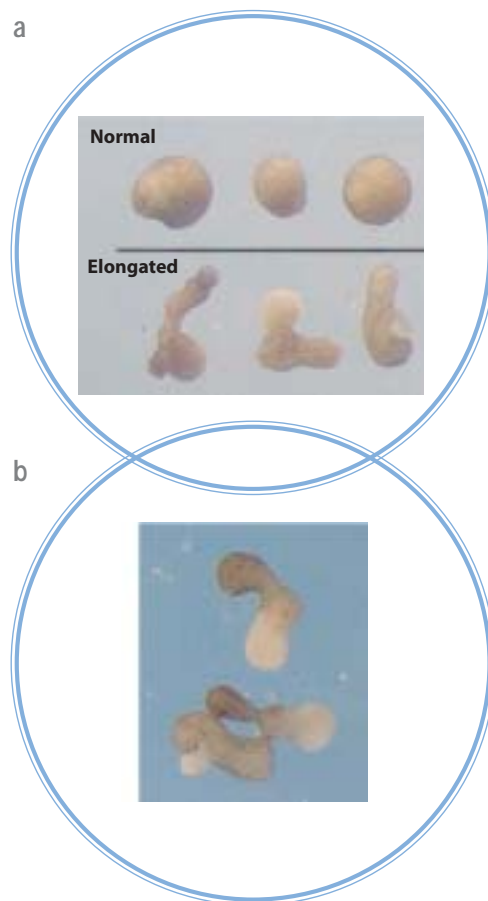
Animal cap explants were removed from stage 8–9 *Xenopus Laevis* embryos and then incubated overnight with rhActivin A, rmGDF-8, rmGDF-5, rmGDF-6, rmGDF-7, or buffer.

a Activin A (0.25 ng/mL) causes elongation of animal caps; explants incubated in buffer alone show no effect.

b GDF-8 (25–100 ng/mL) shows elongation similar to Activin A. GDF-5, -6, and -7 do not produce an elongation effect (data not shown).

“Growth and Differentiation Factor 8 (GDF-8) induces dorsal mesoderm formation in *Xenopus* explants and stimulates erythroid differentiation of K562 human myelogenous leukemia cells.”

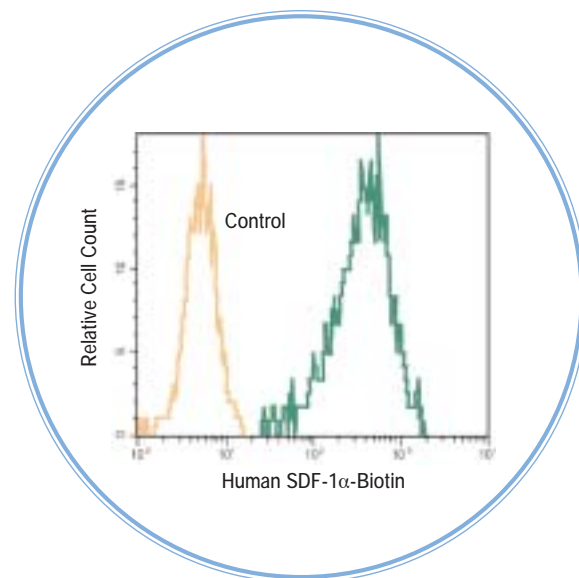
E. Carter, R. Hao, E. Etter, H. Lellman, M. Tsang and M. Breitenfeld. R&D Systems, Inc., Minneapolis, MN, USA.



Applications – Binding Assays

A protein's receptor binding profile can be determined either in a functional ELISA or cell-based systems. Fluorokine® Kits, a flow cytometry application, make use of the specificity of receptor-ligand interactions as an alternative approach to determining the frequency of cells expressing functional cytokine receptors. Cytokines are provided as either biotin- or phycoerythrin-conjugates. Biotinylated cytokines make use of an amplification system that allows multiple avidin-FITC reporter molecules to react with the cell-bound cytokine resulting in brighter signal intensity than conventional staining. Fluorokine® Kits are compatible with fluorescent antibodies, enabling simultaneous, multiparameter analysis of cells. They are ideally suited to the detection of subpopulations of cells that are differentially responsive to cytokines.

Reactivity of Jurkat cells stained with the Human Biotinylated SDF-1 α Fluorokine® Kit (Catalog # NNS00) or Biotinylated Negative Control Reagent (included in the Fluorokine® Kit).



Applications – Standards & Controls

Recombinant proteins can be used as standards and controls for Western blotting, ELISA and many other applications.

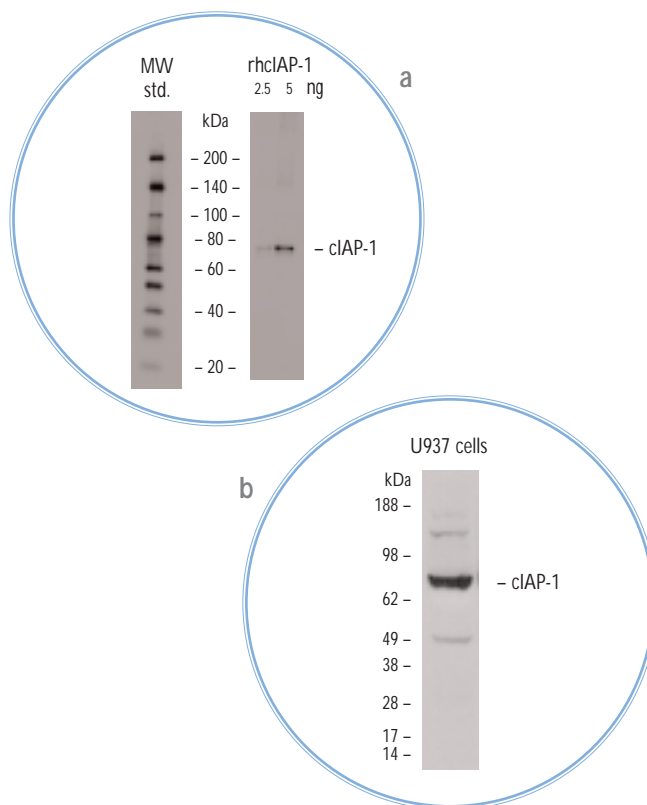
Standards

When used in conjunction with a matched antibody pair, recombinant proteins are used to construct standard curves of known protein concentration from which sample values can be derived. The mass of protein is determined by A_{280} and/or by a coomassie assay using BSA as a standard. After reconstitution, it is advisable to aliquot the protein into appropriate vial sizes to maximize protein stability during storage. The specific activity of a protein does not affect its use in an ELISA. Some proteins, for which matched antibody pairs are available, have no demonstrable biological effect and are only available as ELISA standards, e.g. recombinant human Agouti-related protein (Cat. # 704-AG-002).

Controls

Recombinant proteins can be used as positive controls in Western blots. It is important to remember that the apparent molecular weight of a non-glycosylated, *E. coli*-derived recombinant protein may be different from that of the native protein. The specific activity of a protein does not affect its use in this application. Some proteins used to derive antibodies have no demonstrable biological activity. These proteins are available as Western blot controls.

Recombinant proteins may also be used to pre-absorb antibodies for specificity testing of antibodies in many applications, e.g. immunohistochemistry.



a Recombinant mouse cIAP-1 (a Tic-His fusion protein of ~72.3 kDa) was electrophoresed on a 5–15% gradient polyacrylamide gel, transferred to Immobilon membranes (Millipore), and Western blotted with 0.5 µg/mL rabbit anti-cIAP-1 antibody and visualized using an ECL system. A 30 second exposure is shown.

b Lysate from 5×10^5 U937 cells was electrophoresed on a 4–12% gradient Tris-bis precast gel and Western blotted with 1.0 µg/mL rabbit anti-cIAP-1 antibody. Courtesy of Dr. Jun Kuai, Ph.D, Wyeth Research, 20 Cambridge Park Drive, Cambridge, MA 02140, USA

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Selected Recent References

Descamps, S. *et al.* (2001) *Nerve Growth Factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways.* J. Biol. Chem. **276**:17864–17870.

Kawakami, S. *et al.* (2002) *Paracrine regulation of FSH by follistatin in folliculostellate cell-enriched primate pituitary cell cultures.* Endocrinology **143**:2250–2258.

Puleo, D.A. *et al.* (2002) *A technique to immobilize bioactive proteins, including bone morphogenetic protein-4 (BMP-4), on titanium alloy.* Biomaterials **23**:2079–2087.

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