ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Luciferase Protein Antibodies

☐ **Cat.** LUCF12-A	Rabbit Anti-Luciferase IgG	**SIZE**: 100 ul
☐ **Cat.** LUCF12-BTN	Rabbit Anti-Luciferase IgG-Biotin conjugate	**SIZE**: 500 ul
☐ **Cat.** LUCF12-HRP	Rabbit Anti-Luciferase IgG-HRP conjugate	**SIZE**: 500 ul
☐ **Cat.** LUCF12-FITC	Rabbit Anti-Luciferase IgG-FITC conjugate	**SIZE**: 500 ul
☐ **Cat.** LUCF12-C	Recombinant purified Luciferase protein WB +ve control	**SIZE**: 100 ul

Expression of genes in E. coli or yeast or baculovirus offers a convenient system to produce large amounts of recombinant proteins that may otherwise be difficult to isolate from natural cells and tissues. Very often antibodies to these newly identified proteins are not available to study its biochemical properties, monitor protein expression, and purification. In order to circumvent this problem, short pieces of well-defined peptides (Poly-His, Flag-epitope or c-myc epitope or HA-tag) or small proteins (bacterial GST, MBP, Thioredoxin, b-Galactosidase, VSV-Glycoprotein etc) are often cloned along with the target gene. Proteins are expressed as fusion proteins. Antibodies to these fusion-tags are already available to monitor fusion protein expression and purification. Therefore, fusion-tags serve as universal tags much like secondary antibodies. Many tags have their own characteristics. Poly-His-fusion proteins (6 x His) can bind to Nickel-Sepharose or Nickel-HRP. GST-fusion proteins can bind to glutathione-Sepharose. Therefore, a high degree of purification of fusion protein can be achieved in just one affinity purification step. Purity of fusion proteins can be followed by Tag-antibodies. Very often, fusion proteins are directly injected into animals to generate antibodies. Some fusion tags can be removed later by treatment with enzymes to generate tag-free recombinant proteins.

Source of Antigen and Antibodies

Luciferase was expressed in E. coli. It is ~100-120 kda (a dimer of two subunits of ~50-60 kda). Purified Firefly (Photinus pyralis) Luciferase protein was injected into Rabbit to produce high titer polyclonal antibodies. Antibodies are presented as semipurified IgG (**Cat # LUCF12-A**) in PBS, pH 7.4 and 0.05% azide in liquid or powder form.

Cat# LUCF12-BTN

Purified antibody was coupled to Biotin using ADI Biotinylation kit # 80300 at F/P ratio ~10-20:1. The antibody is supplied in PBS, pH 7.4, 0.2% BSA and 0.05% azide in either **lyophilized** (100 ul) or **liquid** form (100 ul). Reconstitute powder in PBS in 0.5 ml to prepare 1 mg/ml solution. Store at -20oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Purified **Luicferase Protein (**Photinus pyralis, >95%, ~60 kda) for Western blot +ve control (**Cat # LUCF12-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **LUCF12**-C for good visibility with antibody Cat # **LUCF12**-A. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **LUCF12**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Cat# LUCF12-HRP

Purified antibody was coupled to HRP (RZ>3.0) using periodate method. The molar enzyme to protein (E/P) ratio = 4.0. The antibody is supplied in stabilizing buffer, 0.1% prolcin-300 as preservative in either **lyophilized** (0.1 ml) or **liquid** form (100 ul). Reconstitute powder in PBS in 1 ml. Store at 4oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Suggested conjugate dilutions are 1:1,000-1:10,000 ELISA, 1:1K-1:5K for western, and 1:200-1:1000 (IHC).

Cat# LUCF12-FITC

Purified antibody was coupled to FITC at F/P ratio ~3:7. The antibody is supplied in PBS, pH 7.4, 0.2% BSA and 0.05% azide in either **lyophilized** (0.5 ml) or **liquid** form (0.5 ml). Reconstitute powder in PBS in 0.5 ml to prepare stock solution. Store at -20oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Suggested conjugate dilutions are 1:200-1:2000 for immunofluorescence.
Absorption Wavelength: 495 nm
Emission Wavelength: 528 nm

Form & Storage of Antibodies

Store frozen in suitable aliquots. Avoid freeze and thaw. Do not store diluted solutions of antibodies and avoid freeze and thaw. Reconstitute powder in 100 ul PBS by soaking the antibody solution for 10-15 at room temp and light vortexing. Any particulate matter should be removed by centrifugation (10, 000 rpm, 5 min at 4oC). Store liquid in suitable aliquots at –20oC or below.

Recommended Usage

Anti-luciferase IgG have been tested by ELISA (suggested dilution 1K-10K) using 50-100 ng/well luciferase-coated ELISA plates. It recognizes both native and denatured fusion proteins in Western blots (suggested dilution 1:500-1:1K). Actual dilution of antibody will vary depending upon the type of technique used to detect primary antibody such calorimetric or chemiluminescence substrates.

Specificity of Antibodies

The antibodies react with Luciferase (firefly) in ELISA and Western. No significant reactivity is observed with Sea pansy (Renilla reniformis) luciferase.

This product is for in vitro research use only.

Related material available from ADI

Purified Luciferase, Rabbit and Goat and Mab to luciferase

Western blot Recycling Kit; Strips antibodies in 5-10 min at room temp.

LUCF12 150226P

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