

# Affinity-purified Goat Anti-human/mouse PGC1 $\beta$ Antibody

## ORDERING INFORMATION

**Catalog Number:** AF5656

**Lot Number:** XRF01

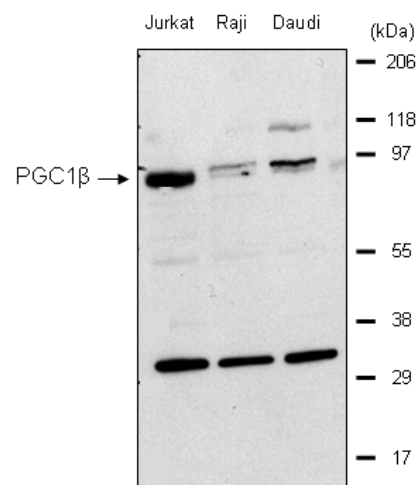
**Size:** 100  $\mu$ g

**Specificity:** human/mouse PGC1 $\beta$

**Immunogen:** *E. coli*-derived rh PGC1 $\beta$   
(aa 315 - 420)

**Ig Type:** goat IgG

**Application:** Western blot



### Detection of PGC1 $\beta$ with AF5656.

30  $\mu$ g of whole cell extracts from exponentially growing Jurkat, Raji, and Daudi cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.2  $\mu$ g/mL goat anti-PGC1 $\beta$  antibody, as described in *Protocols for Immunoblotting*.

## Background

PGC1 $\beta$  (peroxisome proliferator-activated receptor gamma coactivator 1 beta), also known as PPARGC1B, is an 110 kDa protein that belongs to a family of PPAR coactivators. It coactivates nuclear receptors such as ERR $\alpha$ , upregulating expression of proteins that promote mitochondrial fusion and control basal mitochondrial biogenesis. N- and C-terminal alternate sequences, or deletion of amino acids (aa) 156 - 194, produce isoforms of 984, 1017, 1023 (most common) and 1055 aa. Human PGC1 $\beta$  aa 315 - 420, which is common to all isoforms, shares 79% and 76% aa identity with mouse and rat PGC1 $\beta$ , respectively.

## Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human PGC1 $\beta$  (rhPGC1 $\beta$ ; aa 315 - 420; Accession # Q86YN6). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

## Formulation

Lyophilized from a 0.2  $\mu$ m filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute in PBS containing 0.02% NaN<sub>3</sub>.

## Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a **manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

## Specificity

This antibody detects endogenous human/mouse PGC1 $\beta$  in Western blot with an approximate molecular weight of 95 kDa.

## Application

**Western blot** - An antibody concentration of 0.2  $\mu$ g/mL is recommended.

### Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane 1 hour at room temperature in Antibody Solution containing 0.2  $\mu$ g/mL goat anti-human PGC1 $\beta$ .
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

**Cell lysates for Western blottings** - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. 1 mL of boiling 1% SDS lysis buffer (1% SDS, 10 mM Tris-HCl, pH 7.4, 1 mM sodium ortho-vanadate) is added to the plate. The plate is then scraped and the lysis is collected, sonicated and quantified. 30  $\mu$ g of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS-PAGE gel.

**Optimal dilutions should be determined by each laboratory for each application.**