

## Product Datasheet

# GAPDH Antibody (HRP conjugated)

Catalog No: AB2000

Reactivity: H, R, M, Mk, Ch, Ze, Fish

Isotype: Rabbit IgG

Applications: WB



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### Information

**UniProt ID:** P04406

**All Names:** aging-associated gene 9 protein; G3P; G3PD; GAPD; GAPDH; glyceraldehyde 3-phosphate dehydrogenase; Glyceraldehyde-3-phosphate dehydrogenase; MGC88685

**Form:** Liquid

**Storage instructions:** Store at +4°C short term. Store at -20°C long term. Avoid freeze / thaw cycle.

**Storage buffer:** pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.

**Purity:** Affinity-chromatography

**Immunogen:** A synthesized peptide

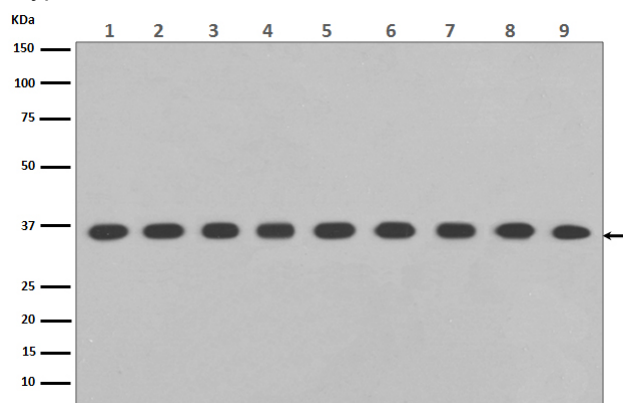
**Molecular Wt.:** 36 kDa

### Application

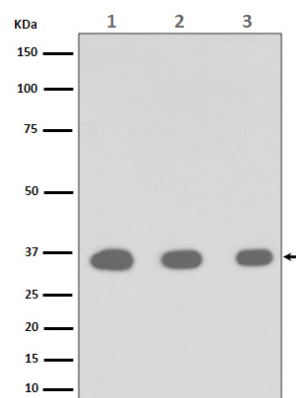
WB 1:5000~1:20000

### Background

Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) is well known as one of the key enzymes involved in glycolysis. GAPDH is constitutively abundant expressed in almost cell types at high levels, therefore antibodies against GAPDH are useful as loading controls for Western Blotting. Some pathology factors, such as hypoxia and diabetes, increased or decreased GAPDH expression in certain cell types.



Western blot analysis of GAPDH expression in (1) Jurkat cell lysate; (2) A375 cell lysate; (3) Human hippocampus lysate; (4) Human fetal liver lysate; (5) COS-1 cell lysate; (6) Raw264.7 cell lysate; (7) Mouse kidney lysate; (8) PC-12 cell lysate; (9) Rat brain lysate.



Western blot analysis of mouse stomach lysates with GAPDH Antibody  
(1)WB:1:5000;(2)1:10000;(3)1:20000

**For Research Use Only. Not For Use In Diagnostic Procedures.**

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## WB(HRP交联抗体用)操作步骤

### 操作流程

#### WB(HRP直标一抗用)操作步骤

1. 常规SDS-PAGE电泳，转印后的PVDF膜，用TBST漂洗掉电转液后，直接放入含有2-5%脱脂奶粉的TBST中（脱脂奶粉要充分溶解在TBST中，不能有奶粉颗粒存在。判断方法：在深色实验台面上，倒少量清水，把脱脂奶粉-TBST溶液加一点到台面的清水中，看是不是溶液状态还是有细小的未溶解奶粉颗粒）。封闭时间：室温1h。封闭时摇床转速不要太快，以液面微微有晃动为准。
2. 封闭结束后，用TBST清洗3次，每次10min。摇床转速要让液面有明显晃动。
3. 第三次TBST清洗后，室温孵育HRP交联后的一抗 1h。一抗需要提前用含有2-5%脱脂奶粉-TBST稀释好。由于一抗已经交联好HRP酶，所以不要过夜孵育一抗。
4. 一抗抚育后，用TBST清洗3次，每次10min。摇床转速要让液面有明显晃动。
5. 清洗后的膜放在曝光板上（HRP交联一抗不需要再孵育二抗），去除多余的TBST液体，但不要膜变干。加上化学发光底物（底物的A液和B液要在室温放置15-30分钟，之后再混合A液和B液）。底物在膜上反应30秒到1分钟后，去掉多余的ECL底物（操作方法：用镊子把膜垂直夹起，膜上ECL底物液体重力自然下流，然后用滤纸把膜下边缘的多余ECL底物吸掉）。多余的ECL底物会一边发生发光反应一边到处流动，造成背景发黑。在膜上盖上玻璃纸后，用纸巾轻轻擦拭玻璃纸，赶走玻璃纸和膜之间的气泡，进入暗室曝光用X胶片曝光。或者直接用仪器曝光。

注：磷酸化抗体可以考虑用1-5% BSA-TBST代替脱脂奶粉-TBST。

#### TBST配方

1×TBST的终浓度为Tris:0.02mol/L，NaCl:0.1mol/L，吐温-20: 0.1%，pH值为7.4。