

Product Guide:

GAPDH

Glyceraldehyde 3-phosphate dehydrogenase

A GAPDH antibody is primarily used as a loading control for western blot experiments due to its high level constitutive expression. GAPDH is considered a 'house keeping' gene and is key in maintenance of many cell functions. Evidence of equal loading of protein in biological experiments is an imperative step in confirming validity of a result.

Our antibodies offer specific and robust results in western blot and ELISA assays, and our GAPDH proteins are of very high purity. We can provide an added aspect of validity of specificity of our GAPDH antibodies through our antibody/blocking peptide pairs, along with our recommended protocol for use.

Monoclonal Antibodies

10R-G109a GAPDH mAb 10R-G109b GAPDH mAb Matched Pair

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-G109a	GAPDH mAb	Mouse	6C5	IgG1	ELISA, ICC, WB	1 mg

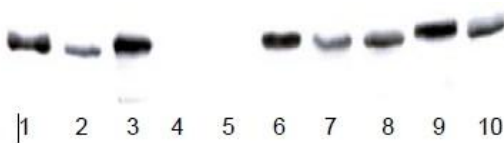
Datasheet <http://www.fitzgerald-fii.com/gapdh-antibody-10r-g109a.html>

10R-G109b	GAPDH mAb	Mouse	4G5	IgG1	ELISA, ICC, Immunoassays, WB	1 mg
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Datasheet <http://www.fitzgerald-fii.com/gapdh-antibody-10r-g109b.html>

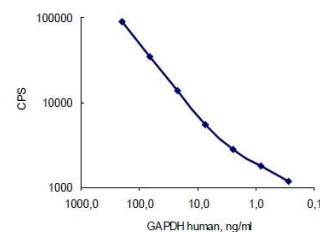
Pairing Information

Matched pair: 10R-G109b can be used as the detection antibody in sandwich immunoassay with 10R-G109a as the capture antibody



Western blot analysis using GAPDH antibody (10R-G109a).

Heart extracts were prepared from different animal species and 0.5 mg of homogenized cardiac tissue was loaded. Anti-mouse IgG (HRP) was used GAPDH immune complex visualization. Lane 1: isolated human GAPDH, 0.5 ug; Lane 2: human heart tissue extract; Lane 3: pig heart tissue extract; Lane 4: goat heart tissue extract; Lane 5: bovine heart tissue extract; Lane 6: dog heart tissue extract; Lane 7: mouse heart tissue extract; Lane 8: rat heart tissue extract; Lane 9: rabbit heart tissue extract; Lane 10: duck heart tissue extract.



ELISA calibration curve for GAPDH antibody (10R-G109a and 10R-G109b).

Antigen: Human GAPDH, Capture: GAPDH antibody (10R-G109a) served as a coating; Detection: GAPDH antibody (10R-G109b) (labelled with stable Eu3+ chelate).

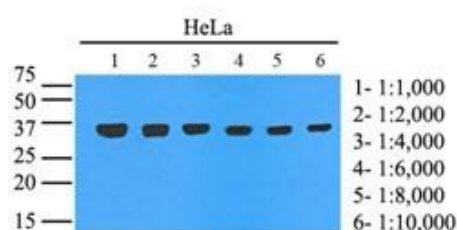
Monoclonal Antibodies

10R-1178 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-1178	GAPDH mAb	Mouse	AT8G4	IgG2b kappa	ELISA, IF, WB	100 ul

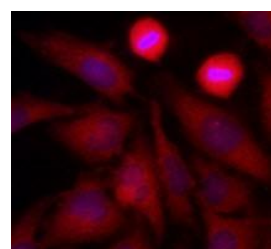
Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-1178.html>



Western Blot analysis of GAPDH antibody (10R-1178)

Cell lysates of HeLa (35 ug) were resolved by SDS-PAGE, transferred to NC membrane and probed with anti-human GAPDH. Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and an ECL detection system.



Immunofluorescent staining using GAPDH antibody (10R-1178)

Immunofluorescence of human HeLa cells stained with Hoechst 3342 (Blue) for nucleus staining and monoclonal anti-human GAPDH antibody (1:500) with Texas Red (Red).

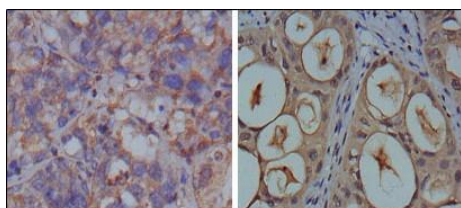
Monoclonal Antibodies

10R- 1931 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-1931	GAPDH mAb	Mouse	1A10	IgG1	ELISA, IF, IHC, WB	100 ul

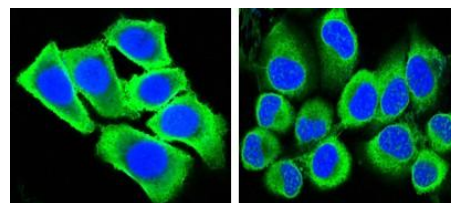
Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-1931.html>



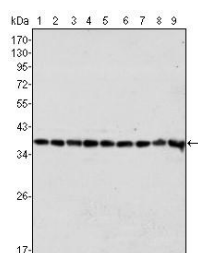
Immunohistochemical staining using GAPDH antibody (10R-1931)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma (left) and kidney carcinoma (right), showing cytoplasmic localization using GAPDH antibody with DAB staining.



Immunofluorescent staining using GAPDH antibody (10R-1931)

Confocal immunofluorescence analysis of methanol-fixed HepG2 (left) and Hela (right) cells using GAPDH antibody (green), showing cytoplasmic localization. Blue: DRAQ5 fluorescent DNA dye.



Western Blot analysis using GAPDH antibody (10R-1931)

Western Blot showing GAPDH antibody used against Hela (1), A549 (2), A431 (3), MCF-7 (4), K562 (5), Jurkat (6), HL60 (7), SKN-SH (8) and SKBR-3 (9) cell lysate.

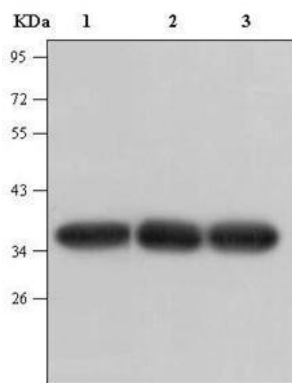
Monoclonal Antibodies

10R- 2685 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-2685	GAPDH mAb	Mouse	1C4	IgG1	WB	100 ul

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-2685.html>



Western Blot analysis using GAPDH antibody (10R-2685)

Western Blot analysis of Hela cell lysate (Lane 1), rat brain tissue lysate (Lane 2) and mouse brain tissue lysate (Lane 3) with GAPDH antibody (1C4) diluted at 1:5000.

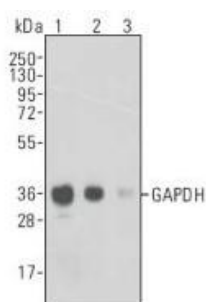
Monoclonal Antibodies

10R- 2687 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-2687	GAPDH mAb	Mouse	3B3	IgG1	IF, WB	100 uI

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-2687.html>



Western Blot analysis using GAPDH antibody (10R-2687)

Western Blot analysis of Hela whole cell lysate using GAPDH Antibody, The dilutions are 1:1000 (Lane 1), 1:2000 (Lane 2), 1:5000 (Lane 3). Each lane was loaded with 20 ug of Hela cell lysate.

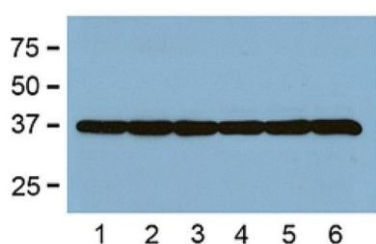
Monoclonal Antibodies

10R- 2932 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-2932	GAPDH mAb	Mouse	GA1R	IgG1	Dot Blot, ELISA, WB	100 ug

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-2932.html>



Western Blot analysis using GAPDH antibody (10R-2932 (1))

1:2000 (0.5 ug/ml) antibody dilution used in WB of 5 ug/lane tissue lysates from human (1), mouse (2), rat (3), rabbit (4), chicken (5) and hamster (6).

10R- 7373 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-7373	GAPDH mAb	Mouse	1D4	IgG1	IF, WB	50ug

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-7373.html>

10R- 7374 GAPDH mAb

Catalog No.	Product	Host	Clone	Isotype	Applications	Size
10R-7374	GAPDH mAb	Mouse	3B3	IgG1	ELISA, IF, WB	50 ul

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-10r-7374.html>

Western Blotting Protocol

Buffers and Reagents:

Wash buffer: PBS (<http://www.fitzgerald-fii.com/pbs-10x-concentrate-85r-125.html>)

Membrane: PVDF Nitrocellulose Membrane

Blocking solution: 5% Skimmed Milk/PBS-T

Wash solution: PBS-T (or TBS-T) PBS or Tris -buffered saline (pH 7.4) with 0.05% (v/v) Tween 20

Primary antibody: See our selection of monoclonals (<http://www.fitzgerald-fii.com/monoclonal-antibodies.html>) or polyclonals at (<http://www.fitzgerald-fii.com/polyclonal-antibodies.html>)

Secondary antibody: See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.

Some other useful reagents: <http://www.fitzgerald-fii.com/diluents.html>

General Procedure:

Sample Preparation

1. Wash the sub-confluent cells with ice-cold PBS.
2. Lyse cells with buffer containing 1% SDS and protease inhibitor.
3. Homogenize and sonicate the cell lysate.
4. Perform a Bradford or Lowry assay to determine the protein concentration levels.
5. Add a loading buffer containing SDS and Beta-ME into the whole cell lysate.
6. Boil the mixture for 5 minutes at 95°C.

Electrophoresis

1. Electrophorese according to standard protocols.
2. Transfer proteins from the gel to a PVDF membrane using an electro blotting apparatus.
3. Incubate the membrane in 5% skim milk/TBS-T for 30 minutes at room temperature.

Immunoblotting

1. Incubate the blocked membrane in primary antibody diluted in 1% skim milk/TBS-T for 1 hour at room temperature. Try a range of dilutions (eg. 0.2-2 (µg/ml) and optimize the dilution according to the results.
2. Wash membrane three times for 5 minutes each time with TBST.
3. Incubate the membrane for 30 minutes at room temperature with HRP conjugated secondary antibody diluted in 1% skim milk/TBST.
4. Wash membrane four times for 5 minutes each time with TBST.
5. Incubate membrane in chemiluminescence reagent and visualize proteins using image analyzer.

Polyclonal Antibodies

20-GS25 GAPDH pAb

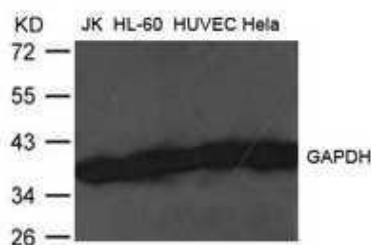
Catalog No.	Product	Host	Applications	Size
20-GS25	GAPDH pAb	Sheep	ELISA, WB	100 ul

Datasheet <http://www.fitzgerald-fii.com/gapdh-antibody-20-gs25.html>

20R-2715 GAPDH pAb

Catalog No.	Product	Host	Applications	Size
20R-2715	GAPDH pAb	Rabbit	WB	50 ug

Datasheet <http://www.fitzgerald-fii.com/gapdh-antibody-20r-2715.html>



Western Blot analysis of extract from JK, HL-60, HUVEC and HeLa cells using GAPDH antibody.

20R-2798 GAPDH pAb

Catalog No.	Product	Host	Applications	Size
20R-2798	GAPDH pAb	Rabbit	IF, WB, IHC	50 ug

Datasheet <http://www.fitzgerald-fii.com/gapdh-antibody-20r-2798.html>

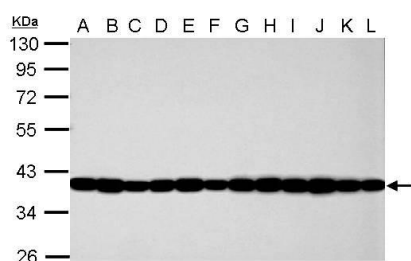
Polyclonal Antibodies

70R-12491 GAPDH pAb

Catalog No.	Product	Host	Applications	Size
70R-12491	GAPDH pAb	Rabbit	IF, ICC, IHC-P, WB	100 ul

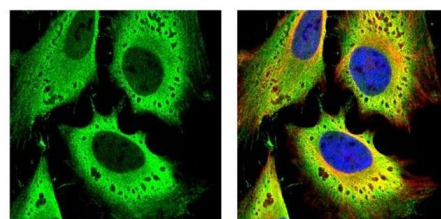
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<http://www.fitzgerald-fii.com/gapdh-antibody-70r-12491.html>



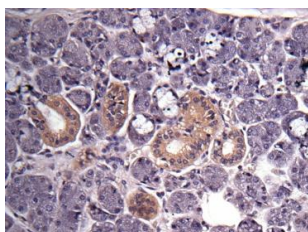
Western blot analysis using GAPDH antibody (70R-12491)

Western blot analysis of 30 ug of whole cell lysate (A: Jurkat; B: Raji; C: 293T; D: A431; E: HeLa; F: HepG2; G: H1299; H: HCT116; I: MCF-7; J: NT2D1; K: PC-3; L: U87-MG) using a 10 % SDS PAGE gel and GAPDH antibody at a dilution of 1:10000 .



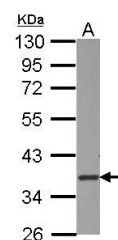
Immunofluorescent staining using GAPDH antibody (70R-12491)

Confocal immunofluorescence analysis (Olympus FV10i) of paraformaldehyde-fixed HeLa, using GAPDH antibody (green) at 1:500 dilution. Alpha-tubulin filaments were labeled with (red) at 1:2000.



Immunohistochemical staining using GAPDH antibody (70R-12491)

Immunohistochemical staining of paraffin-embedded salivary gland cancer tissue using GAPDH antibody at a dilution of 1:500.



Western blot analysis using GAPDH antibody (70R-12491)

Western blot analysis of 30 ug of whole cell lysate (A: drosophila) using a 10 % SDS PAGE gel and GAPDH antibody at a dilution of 1:5000

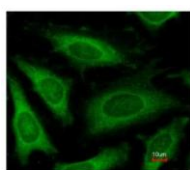
Polyclonal Antibodies

70R-12513 GAPDH pAb

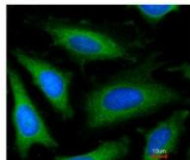
Catalog No.	Product	Host	Applications	Size
70R-12513	GAPDH pAb	Rabbit	IF, ICC, IHC-P, WB	100 ul

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-70r-12513.html>

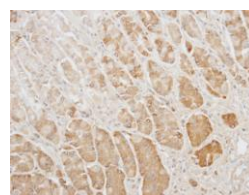


Costained with Hoechst 33342



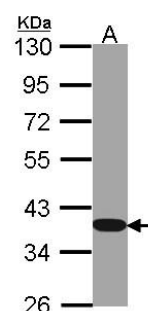
Immunofluorescent staining using GAPDH antibody (70R-12513)

Immunofluorescence analysis of methanol-fixed HeLa, using GAPDH antibody at 1:100 dilution.



Immunohistochemical staining using GAPDH antibody (70R-12513)

Immunohistochemical staining of paraffin-embedded Gastric CA using GAPDH antibody at a dilution of 1:100.



Western blot analysis using GAPDH antibody (70R-12513)

Western blot analysis of 30 ug of whole cell lysate (A: Hep G2) using a 10 % SDS PAGE gel and GAPDH antibody at a dilution of 1:1000.

70R-13880 GAPDH pAb

Catalog No.	Product	Host	Applications	Size
70R-13880	GAPDH pAb	Rabbit	IHC-P, IHC-F, ICC, WB	100 ug

Datasheet

<http://www.fitzgerald-fii.com/gapdh-antibody-70r-13880.html>

Polyclonal Antibodies & Blocking Peptides

70R-2033 GAPDH pAb + 33R-3921 GAPDH Blocking Peptide

Catalog No.	Product	Host	Applications	Size
70R-2033	GAPDH pAb	Rabbit	WB, IHC	50 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-antibody-70r-2033.html			
33R-3921	GAPDH Blocking Peptide	-	WB, IHC	100 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-blocking-peptide-33r-3921.html			



Western Blot analysis using GAPDH antibody (70R-2033)

GAPDH antibody (70R-2033) used at 1 ug/ml to detect target protein.

Polyclonal Antibodies & Blocking Peptides

70R-2145 GAPDH pAb + 33R-4239 GAPDH Blocking Peptide

Catalog No.	Product	Host	Applications	Size
70R-2145	GAPDH pAb	Rabbit	WB, IHC	50 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-antibody-70r-2145.html			
33R-4239	GAPDH Blocking Peptide	-	WB, IHC	100 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-blocking-peptide-33r-4239.html			

90 kDa__
65 kDa__
40 kDa__
31 kDa__
22 kDa__



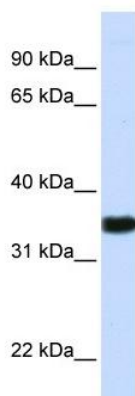
Western Blot analysis using GAPDH antibody (70R-2145)

GAPDH antibody (70R-2145) used at 1 ug/ml to detect target protein.

Polyclonal Antibodies & Blocking Peptides

70R-2150 GAPDH pAb + 33R-4240 GAPDH Blocking Peptide

Catalog No.	Product	Host	Applications	Size
70R-2150	GAPDH pAb	Rabbit	WB, IHC	50 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-antibody-70r-2150.html			
33R-4240	GAPDH Blocking Peptide	-	WB, IHC	100 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-blocking-peptide-33r-4240.html			



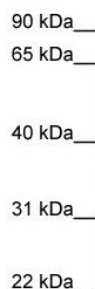
Western Blot analysis using GAPDH antibody (70R-2150)

GAPDH antibody (70R-2150) used at 1 ug/ml to detect target protein.

Polyclonal Antibodies & Blocking Peptides

70R-2187 GAPDH pAb + 33R-4241 GAPDH Blocking Peptide

Catalog No.	Product	Host	Applications	Size
70R-2187	GAPDH pAb	Rabbit	WB, IHC	50 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-antibody-70r-2187.html			
33R-4241	GAPDH Blocking Peptide	-	WB, IHC	100 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-blocking-peptide-33r-4241.html			



90 kDa
65 kDa
40 kDa
31 kDa
22 kDa

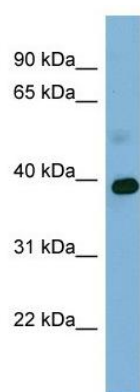
Western Blot analysis using GAPDH antibody (70R-2187)

GAPDH antibody (70R-2187) used at 1 ug/ml to detect target protein.

Polyclonal Antibodies & Blocking Peptides

70R-2302 GAPDH pAb + 33R-4242 GAPDH Blocking Peptide

Catalog No.	Product	Host	Applications	Size
70R-2302	GAPDH pAb	Rabbit	WB, IHC	50 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-antibody-70r-2302.html			
33R-4242	GAPDH Blocking Peptide	-	WB, IHC	100 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-blocking-peptide-33r-4242.html			



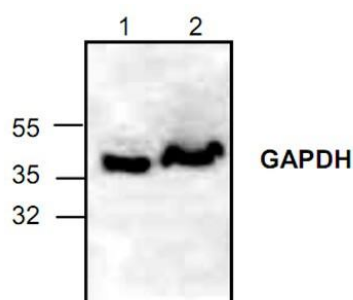
Western Blot analysis using GAPDH antibody (70R-2302)

GAPDH antibody (70R-2302) used at 1 ug/ml to detect target protein.

Polyclonal Antibodies & Blocking Peptides

70R-12079 GAPDH pAb + 33R-10915 GAPDH Blocking Peptide

Catalog No.	Product	Host	Applications	Size
70R-12079	GAPDH pAb	Rabbit	WB, IHC	100 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-antibody-70r-12079.html			
33R-10915	GAPDH Blocking Peptide	-	WB, IHC	50 ug
Datasheet	http://www.fitzgerald-fii.com/gapdh-blocking-peptide-33r-10915.html			



Western Blot analysis using GAPDH antibody (70R-12079)

Western blot analysis of GAPDH in Jurkat cell lysate (Lane 1 & 2).

Using Blocking Peptides as Controls in Western Blotting and IHC

To determine which band or staining is specific, an immunizing peptide blocking experiment can be performed. Before carrying out the staining of the immunoblot, the antibody is neutralized (incubated with an excess of peptide that corresponds to the epitope recognized by the antibody). The antibody that is bound to the blocking peptide can no longer bind to the epitope present in the protein on the Western blot. The neutralized antibody is then used side-by-side with the antibody alone, and the results are compared. By comparing the staining from the blocked antibody versus the antibody alone, you can see which staining is specific. A similar principal can be employed for IHC using two slides.

Buffers and Reagents:

- Blocking buffer of choice (usually TBST plus either 5% non-fat dry milk or 3% BSA for Western blot, or PBS plus 1% BSA for IHC)
- Primary antibody: See our selection of monoclonals at <http://www.fitzgerald-fii.com/monoclonal-antibodies.html> or polyclonals at <http://www.fitzgerald-fii.com/polyclonal-antibodies.html>
- Secondary antibody: See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.
- Blocking peptide: (corresponding to the epitope recognized by the antibody)
- Two identical samples
- Western blot - two identical lanes, cut in half
- IHC - two slides containing the cells/tissue of interest

General Procedure:

1. Determine the optimal concentration of antibody that consistently gives a positive result in your particular protocol. Using that concentration, determine how much antibody you will need for two experiments. For example, an antibody is being used successfully in Western blot at 0.5 µg/ml. You will need 2 ml of antibody solution to stain one strip of a Western blot. Thus, you would use 1 µg of antibody in 2 ml buffer for each strip.
2. Dilute the necessary amount of antibody in blocking buffer to the final volume needed for the two experiments. Divide this equally into two tubes.
3. Label one tube '+ BP' and add peptide to a final concentration of 1 µg/ml (2 µg total peptide in this example).
4. Label the other tube '-BP' and add an equivalent amount of buffer.
5. Incubate both tubes, with agitation, at room temperature for 30 minutes, or overnight at 4°C.
6. Perform the staining protocol on the two identical samples, using the blocked antibody for one and the control for the other. Observe the staining. The staining that disappears when using the blocked antibody is specific to the antibody. (If more than one band disappears in Western blot by peptide competition, those bands contain the antigenic determinants and could be fragments of the full antigen or a complex containing the antigen.

Proteins

30R-AG018 GAPDH protein

Catalog No.	Product	Size
30R-AG018	GAPDH protein	100 ug

Datasheet <http://www.fitzgerald-fii.com/gapdh-protein-30r-ag018.html>

30R-AG005 GAPDH protein

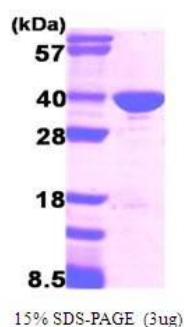
Catalog No.	Product	Size
30R-AG005	GAPDH protein (Rabbit)	100 ug

Datasheet <http://www.fitzgerald-fii.com/gapdh-protein-rabbit-30r-ag005.html>

30R-1193 GAPDH protein

Catalog No.	Product	Size
30R-1193	GAPDH protein	100 ug

Datasheet <http://www.fitzgerald-fii.com/gapdh-protein-rabbit-30r-1193.html>



*Coomassie Blue stained SDS-PAGE of GAPDH protein
(30R-1193)*

*Figure annotation denotes ug of protein loaded and %
gel used.*



Ordering Information

All items can be ordered:

- Online at www.fitzgerald-fii.com
- By telephoning our Customer Service department on +978.371.6446
- By e-mail to antibodies@fitzgerald-fii.com

Phone lines are open from 9am to 6pm EST. Online orders can be placed at any time and will be processed during office hours.