

## Product datasheet

# Anti-Hexokinase II antibody [EPR20839] ab209847

**KO VALIDATED** Recombinant RabMAB

★★★★★ **2 Abreviews** **74 References** **12 Images**

### Overview

<b>Product name</b>	Anti-Hexokinase II antibody [EPR20839]
<b>Description</b>	Rabbit monoclonal [EPR20839] to Hexokinase II
<b>Host species</b>	Rabbit
<b>Specificity</b>	Not recommended for rat and mouse tissues in IHC.
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: Hexokinase II recombinant protein (aa16-458); HEK-293, HCT116, HeLa, SH-SY5Y and NIH/3T3 whole cell lysates; HKDC1 and Hexokinase I-knockdown HeLa cell lysates; Rat testis and muscle lysates; Human testis, colon and fetal heart lysates; Mouse muscle lysate. IHC-P: Human heart and pancreatic adenocarcinoma tissues. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAB<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAB<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR20839
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab209847 in the following tested applications.

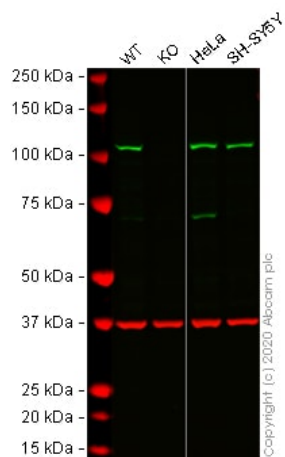
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
<b>WB</b>	★★★★★ (2)	1/1000. Detects a band of approximately 102 kDa (predicted molecular weight: 102 kDa).
<b>IHC-P</b>		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Not recommended for rat and mouse tissues.
<b>ICC/IF</b>		1/100.
<b>IP</b>		1/30.
<b>Flow Cyt (Intra)</b>		1/60.

## Target

<b>Tissue specificity</b>	Predominant hexokinase isozyme expressed in insulin-responsive tissues such as skeletal muscle.
<b>Pathway</b>	Carbohydrate metabolism; hexose metabolism.
<b>Sequence similarities</b>	Belongs to the hexokinase family. Contains 2 hexokinase domains.
<b>Domain</b>	The N- and C-terminal halves of this hexokinase show extensive sequence similarity to each other. The catalytic activity is associated with the C-terminus while regulatory function is associated with the N-terminus. Each domain can bind a single glucose and Gluc-6-P molecule.
<b>Cellular localization</b>	Mitochondrion outer membrane. Its hydrophobic N-terminal sequence may be involved in membrane binding.

## Images



Western blot - Anti-Hexokinase II antibody  
[EPR20839] (ab209847)

**All lanes** : Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/1000 dilution

**Lane 1** : Wild-type HCT116 cell lysate

**Lane 2** : HK2 knockout HCT116 cell lysate

**Lane 3** : HeLa cell lysate

**Lane 4** : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

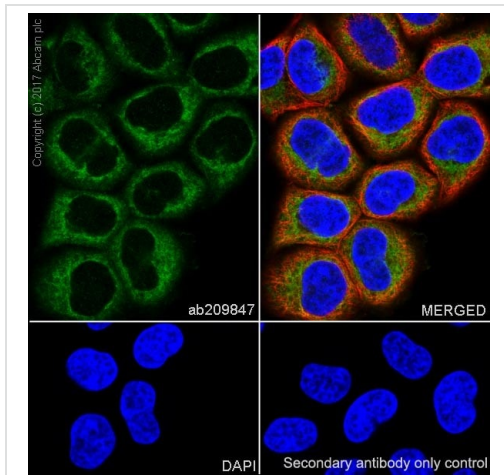
Performed under reducing conditions.

**Predicted band size:** 102 kDa

**Observed band size:** 105 kDa

**Lanes 1 -4:** Merged signal (red and green). Green - ab209847 observed at 105 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab209847 was shown to react with Anti-Hexokinase II in HCT 116 wild-type cells in western blot with loss of signal observed in HK2 knockout cell line **ab273721** (HK2 knockout cell lysate **ab275250**). HCT 116 wild-type and HK2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween<sup>®</sup>) before incubation with ab209847 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

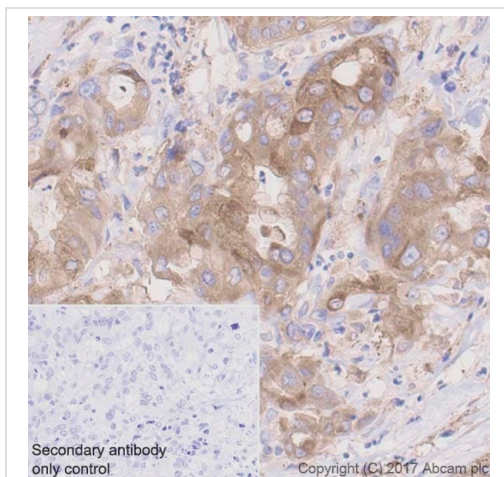


Immunocytochemistry/ Immunofluorescence - Anti-Hexokinase II antibody [EPR20839] (ab209847)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Hexokinase II with ab209847 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).  
Cytoplasmic staining in HeLa cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 dilution.

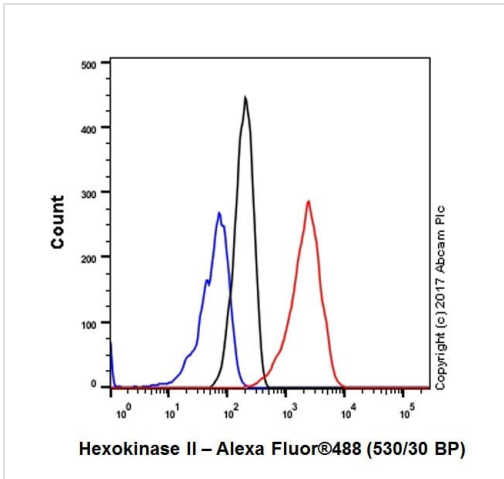


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hexokinase II antibody [EPR20839] (ab209847)

Immunohistochemical analysis of paraffin-embedded human pancreatic adenocarcinoma tissue labeling Hexokinase II with ab209847 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Strong cytoplasmic staining in human pancreatic adenocarcinoma (PMID: 26137268) is observed. Counter stained with Hematoxylin.

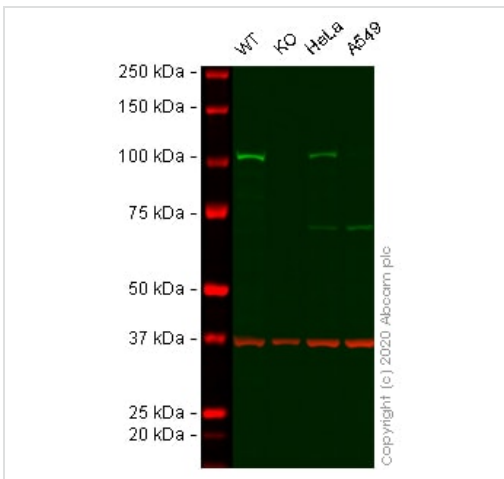
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Hexokinase II antibody [EPR20839] (ab209847)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Hexokinase II with ab209847 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control ([ab172730](#)) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-Hexokinase II antibody [EPR20839] (ab209847)

**All lanes** : Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/1000 dilution

**Lane 1** : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 2** : HK2 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 3** : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 4** : A549 (Human lung carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

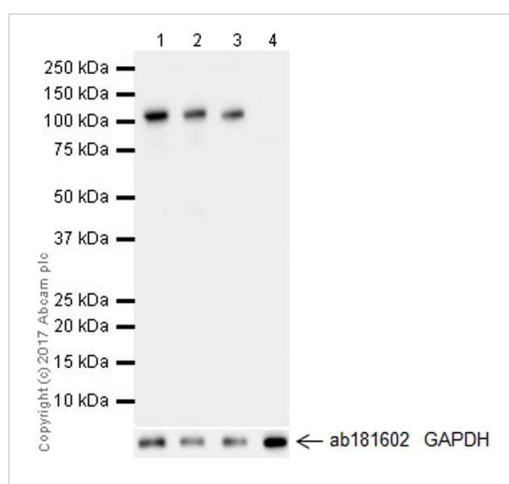
**Predicted band size:** 102 kDa

**Observed band size:** 110 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab209847

observed at 110 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab209847 was shown to react with Hexokinase II in wild-type HEK-293 cells in western blot with loss of signal observed in HK2 knockout sample. Wild-type and HK2 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab209847 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Hexokinase II antibody  
[EPR20839] (ab209847)

**All lanes** : Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/1000 dilution

**Lane 1** : HeLa (human epithelial cell line from cervix adenocarcinoma), whole cell lysate

**Lane 2** : HKDC1-knockdown HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate

**Lane 3** : Hexokinase I-knockdown HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate

**Lane 4** : Hexokinase II-knockdown HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

**Predicted band size:** 102 kDa

**Observed band size:** 102 kDa

**Exposure time:** 41 seconds

Blocking/Dilution buffer: 5% NFDN/TBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP

instrument.

Hexokinase II was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab209847 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab209847 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/10000 dilution.

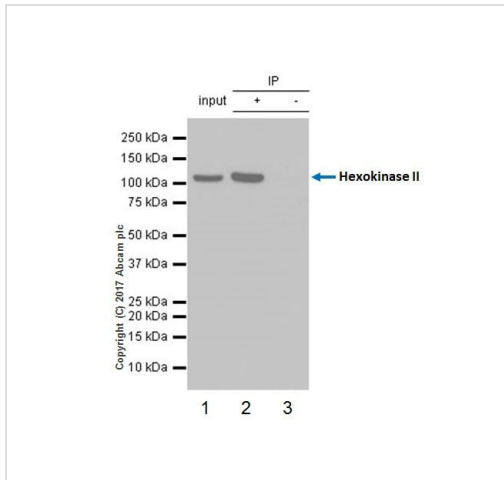
**Lane 1:** HeLa whole cell lysate 10 µg (Input).

**Lane 2:** ab209847 IP in HeLa whole cell lysate.

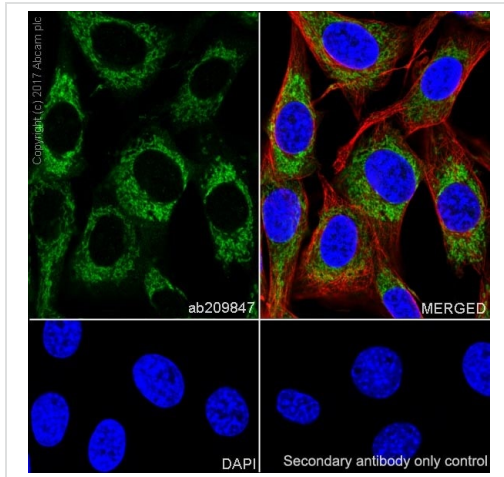
**Lane 3:** Rabbit monoclonal IgG ([ab172730](#)) instead of ab209847 in HeLa whole cell lysate.

**Exposure time:** 30 seconds.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.



Immunoprecipitation - Anti-Hexokinase II antibody [EPR20839] (ab209847)

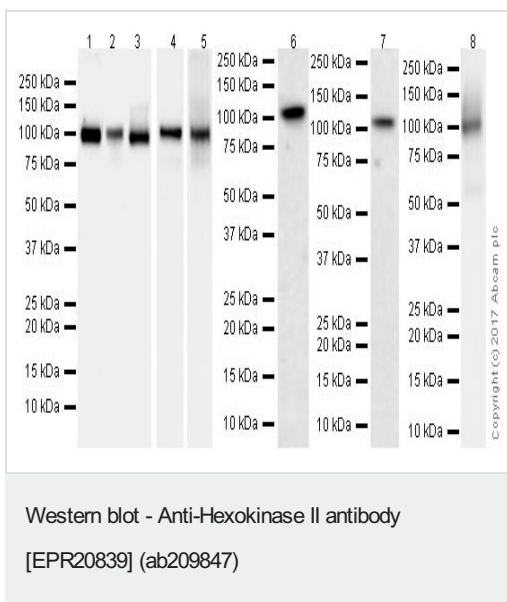


Immunocytochemistry/ Immunofluorescence - Anti-Hexokinase II antibody [EPR20839] (ab209847)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling Hexokinase II with ab209847 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) ([ab150077](#)) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining in NIH/3T3 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) ([ab195889](#)) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.



**All lanes** : Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/1000 dilution

**Lane 1** : Rat testis lysate at 20 µg

**Lane 2** : Rat muscle lysate at 20 µg

**Lane 3** : SH-SY5Y (human neuroblastoma cell line from bone marrow), whole cell lysate at 20 µg

**Lane 4** : Human testis lysate at 20 µg

**Lane 5** : Mouse muscle lysate at 20 µg

**Lane 6** : Human colon lysate at 10 µg

**Lane 7** : Human fetal heart lysate at 10 µg

**Lane 8** : NIH/3T3 (mouse embryonic fibroblast cell line), whole cell lysate at 10 µg

### Secondary

**Lanes 1-5 & 8** : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

**Lanes 6-7** : VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) at 1/4000 dilution

Developed using the ECL technique.

**Predicted band size:** 102 kDa

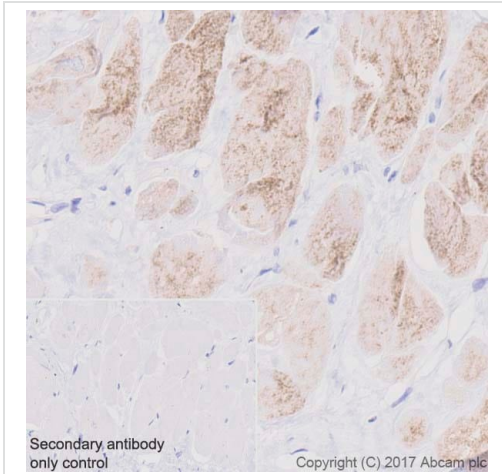
**Observed band size:** 102 kDa

**Exposure times:** Lanes 1-3,6,7: 3 minutes; Lanes 4,5,8: 58 seconds.

Blocking/Dilution buffer: 5% NFD/MTBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.



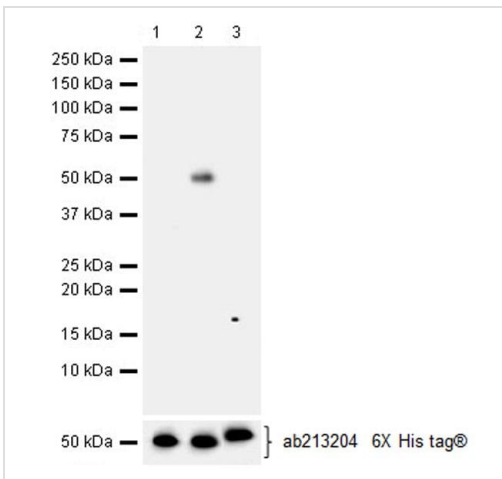


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hexokinase II antibody [EPR20839] (ab209847)

Immunohistochemical analysis of paraffin-embedded human heart tissue labeling Hexokinase II with ab209847 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Granularly cytoplasmic staining in human heart (PMID: 4058069; PMID: 26722360) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Hexokinase II antibody [EPR20839] (ab209847)

**All lanes** : Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/10000 dilution

**Lane 1** : HKDC1 recombinant protein (aa16-458)

**Lane 2** : Hexokinase II recombinant protein (aa16-458)

**Lane 3** : Hexokinase I recombinant protein (aa16-458)

Lysates/proteins at 0.01 µg per lane.

#### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

**Predicted band size:** 102 kDa


**Observed band size:** 50 kDa

**Exposure time:** 3 minutes

Blocking/dilution buffer and concentration: 5% NFD/MTBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.

Why choose a recombinant antibody?



- Research with confidence**  
Consistent and reproducible results
- Long-term and scalable supply**  
Recombinant technology
- Success from the first experiment**  
Confirmed specificity
- Ethical standards compliant**  
Animal-free production

Anti-Hexokinase II antibody [EPR20839] (ab209847)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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