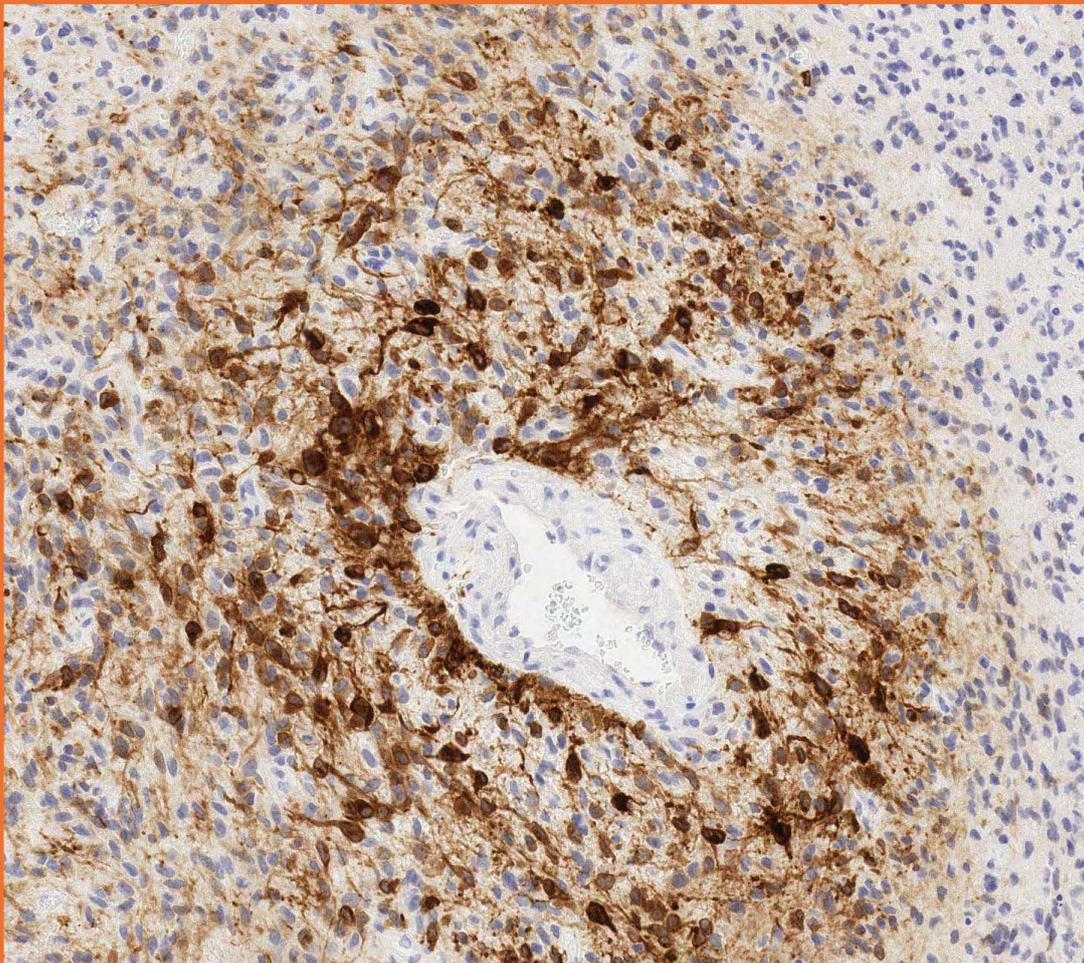


Enhanced validation data

Anti-EGFRvIII recombinant antibody – ab313646



Enhanced validation of Anti-EGFRvIII recombinant antibody [EPR28380-83] – ab313646

Enhanced validation designed for your needs

We understand the challenge of finding the right antibody clone – highly specific and sensitive to your intended target – at early selection stages of your development program. To de-risk this clone selection process for you, we generated enhanced validation data for our best recombinant antibody clones to some of the most promising targets.

Our enhanced validation gives you an extra level of confidence in an antibody clone

- Provides additional data on the specificity and sensitivity of our recombinant antibodies in immunohistochemistry (IHC) and other relevant techniques
- Carried out in a custom manner, specific both to the target and the relevant research & clinical settings
- Builds upon our high-quality standard validation

Our framework for enhanced validation

- Our enhanced validation focuses on generating detailed IHC expression profiles for promising oncology targets in selected formalin-fixed paraffin-embedded (FFPE) human normal tissues and cancer tissue microarrays (TMAs).
- In this study, we demonstrate the sensitivity and specificity of anti-EGFRvIII recombinant antibody (ab313646) in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®) and DISCOVERY ULTRA (Roche Diagnostics).
- A multiplex (duplex) assay was also developed using the DISCOVERY ULTRA (Roche Diagnostics).

Target overview

Approved symbol

EGFRvIII

Approved name

Epidermal Growth Factor Receptor variant III (EGFRvIII)

Chromosomal location:

7p11.2

Function

- Wild-type (EGFR): Key modulator of cell growth that regulates cell proliferation, differentiation, and survival.
- EGFRvIII mutation: A constitutively active, oncogenic EGFR deletion variant lacking the ligand-binding domain (exons 2–7)¹⁻⁴.
 - Observed in 30% of glioblastoma (GBM) cases.
 - By stimulating mitogenic and anti-apoptotic signaling pathways, EGFRvIII enhances the tumorigenic capacity of GBM.

Tissue specificity

- Wild-type (EGFR): membranous and cytoplasmic expression in several tissues; most abundant in placenta.
- EGFRvIII: well demonstrated in glioblastoma and human lung squamous cell carcinoma.

Cellular localization

Cell membrane. Expressed at the cell surface. EGFRvIII RNA and protein can also be transferred to other cells via microvesicles to promote tumorigenesis.

Target information above in part from: UniProt accession [P00533](#)

The UniProt Consortium

The Universal Protein Resource (UniProt) in 2010

[Nucleic Acids Res. 38:D142-D148 \(2010\)](#)

Materials and methods

Anatomic site	Disease
Placenta	Normal
Brain	Cancer

Table 1. List of human FFPE tissues used in the enhanced validation. All tissues were sourced from Abcam-approved tissue suppliers.

Tissue microarray (TMA)	Cores	Cases	Normal/Benign cases	Cancer cases	Source (#catalog number)
Multi-normal	34	34	34	0	Pantomics (#MEL1021)
Nervous system gliomas	96	48	3	45	Pantomics (#NGL961)

Table 2. List of human TMAs used in the enhanced validation. All tissues were sourced from Abcam-approved tissue suppliers.

Step	Reagents	Pre-programmed protocol
Dewax	Bond™ dewax solution (AR922), alcohol, BOND wash solution (AR9590)	Dewax
Antigen retrieval	Bond™ epitope retrieval ER2 solution (AR9640)	HIER with ER2 (pH 9.0), 20 minutes, 100°C

Table 3a. IHC prestaining protocol on BOND™ RX Research Stainer (Leica®).

Leica® is a registered trademark of Leica Microsystems IR GmbH. BOND™ is a trademark of Leica Biosystems Melbourne Pty. Ltd.

Step	Reagents	Number of washes	Time (minutes)
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Anti-EGFRvIII recombinant antibody – [EPR28380-83] – ab313646 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 2.5 µg/mL	-	15
Wash	Bond™ wash solution	3x	0
Secondary antibody	Bond™ polymer refine detection (DS9800)	-	8
Wash	Bond™ wash solution	2x	4
	Deionized water	1x	0
Visualization	Mixed DAB refine (DS9800)	1x	0
	Mixed DAB refine (DS9800)	-	10
Wash	Deionized water	3x	0
Counterstain	Hematoxylin (DS9800)	-	5
Wash	Deionized water	1x	0
	Bond™ wash solution	1x	0
	Deionized water	1x	0

Table 3b. IHC staining protocol on BOND™ RX Research Stainer (Leica®). The protocol used is the same as the default IHC protocol F on BOND™ RX Research Stainer (Leica®), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature.

Step	Reagents	Method
Deparaffinization	EZ Prep	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	64 min, 100 °C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Anti-EGFRvIII recombinant antibody – [EPR28380-83] – ab313646 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 3.0 µg/mL	16 min, 37 °C
Counterstain	Hematoxylin II	8 min
Post counterstain	Bluing reagent	4 min

Table 4. IHC staining protocol on the DISCOVERY ULTRA (Roche Diagnostics) instrument. Staining was performed using standard conditions with OptiView DAB IHC Detection Kit (#760-700).

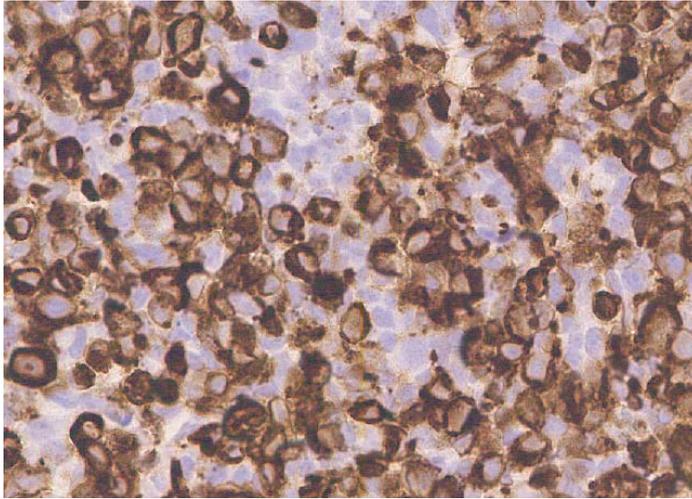
Step	Reagents	Method
Deparaffinization	EZ Prep	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	64 min, 95°C
DISC inhibitor	DISCOVERY Inhibitor (#760-4840)	8 min
1 st Primary antibody	Anti-EGFRvIII recombinant antibody – [EPR28380-83] – ab313646 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 3.0 µg/mL	16 min, 37 °C
1 st Linking antibody	DISCOVERY Anti-Rb HQ (#760-4815)	12 min, 37 °C
1 st Enzyme conjugate	DISCOVERY Anti-HQ HRP (#760-4820)	12 min
1 st HRP-driven chromogen	DISCOVERY Purple kit (RUO) (#760-229)	12 min
Dual sequence-antibody denaturation	ULTRA Cell Conditioning Solution (ULTRA CC2)	8 min, 100 °C
2 nd Primary antibody	Anti-EGFR recombinant antibody – [EP38Y] – ab52894 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 0.5 µg/mL	16 min, 37 °C
2 nd Linking antibody	DISCOVERY Anti-Rb HQ (#760-4815)	12 min, 37 °C
2 nd Enzyme conjugate	DISCOVERY Anti-HQ HRP (#760-4820)	12 min
2 nd HRP-driven chromogen	DISCOVERY Teal kit (RUO) (#760-247) Teal HRP H202	4 min
	DISCOVERY Teal kit (RUO) (#760-247) Teal HRP Act	12 min
Counterstain	Hematoxylin II	4 min

Table 5. Duplex IHC staining protocol on the DISCOVERY ULTRA (Roche Diagnostics) instrument. Staining was performed using standard conditions with DISCOVERY Purple kit (RUO) (#760-229) and DISCOVERY Teal HRP kit (#760-247). These are translucent chromogens that shift in color when both are present in the same cell and sub-cellular compartment. Co-localized DISCOVERY Purple and DISCOVERY Teal combine to form an indigo blue to deep purple color.

EGFRvIII expression in FFPE cell pellets (BOND™ RX)

Below are the representative images of EGFRvIII/EGFR expression in FFPE 293T (human embryonic kidney epithelial) cells. As expected, EGFRvIII was detected in the variant expressing cell line and absent in the wild-type expressing cell line.

293T cells overexpressing EGFRvIII



293T cells overexpressing EGFR

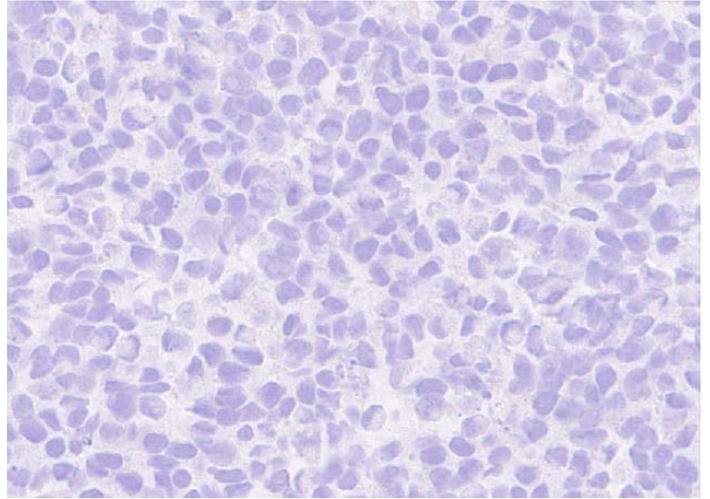


Figure 1. EGFRvIII expression in EGFRvIII and EGFR overexpressing cell lines.

Immunohistochemical analysis of FFPE 293T (human embryonic kidney epithelial cell) transfected with human EGFRvIII expression vector containing a Myc-His tag and human wild-type EGFR expression vector containing a Myc-His tag. Cells transfected with an empty vector showed no staining (data not shown).

IHC images show EGFRvIII detection using anti-EGFRvIII recombinant antibody (ab313646) at a concentration of 5.09 µg/mL. Positive staining in brown; counterstained with hematoxylin.

Differential EGFRvIII and EGFR expression (BOND™ RX)

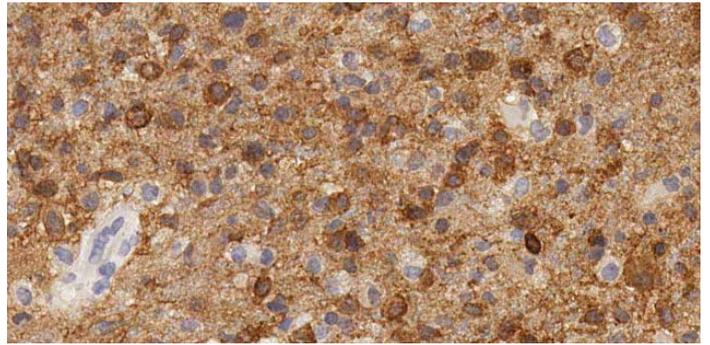
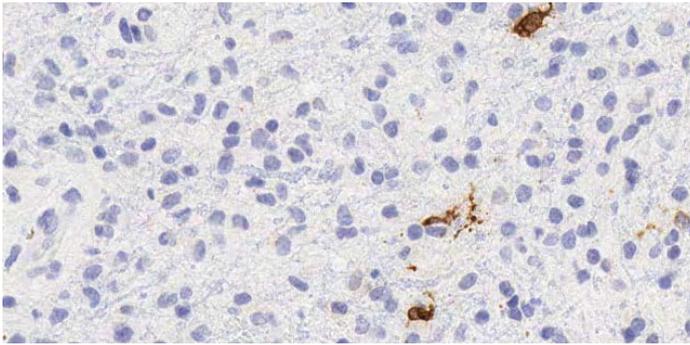
Below are the representative images of glioblastoma and normal placental tissues expressing EGFRvIII and EGFR, demonstrating antibody specificity. EGFRvIII-expressing cell frequency within tissue samples ranged from low to high.

EGFRvIII

EGFR

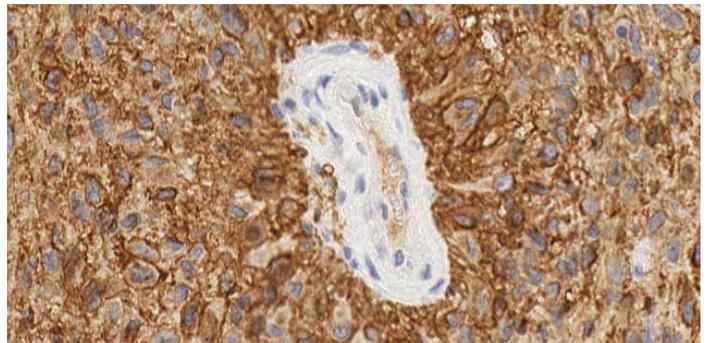
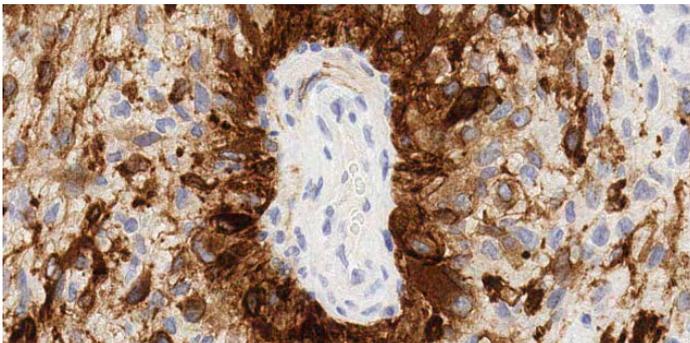
Glioblastoma (low EGFRvIII expression frequency)

Glioblastoma (high EGFR expression)



Glioblastoma (high EGFRvIII expression frequency)

Glioblastoma (high EGFR expression)



Normal placenta (no EGFRvIII expression)

Normal placenta (high EGFR expression)

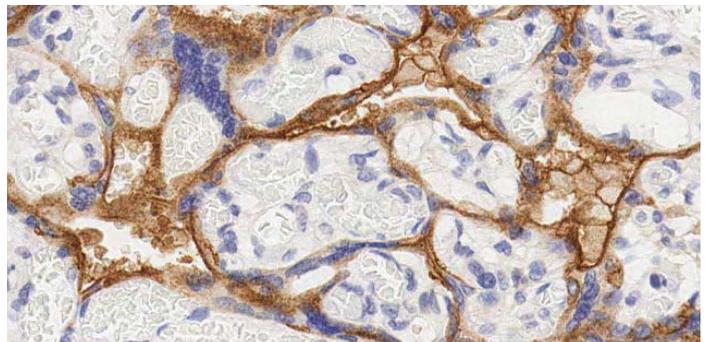
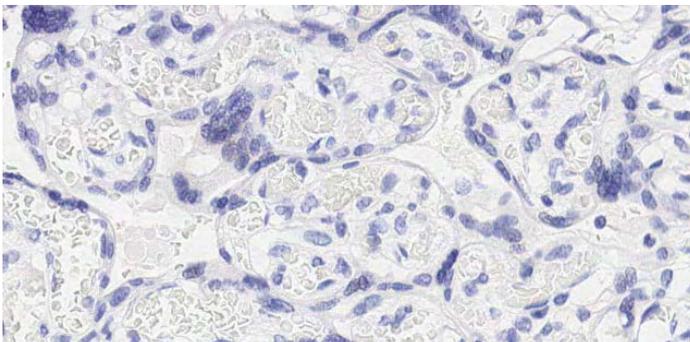


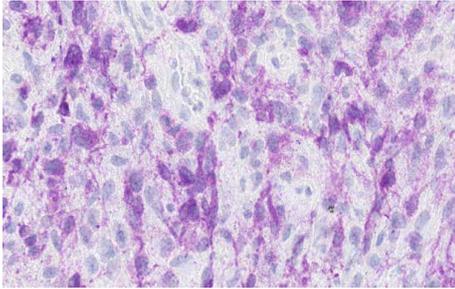
Figure 2. EGFRvIII and EGFR expression in glioblastoma and normal placenta tissues. IHC images show EGFRvIII/EGFR expression using anti-EGFRvIII antibody (ab313646) and anti-EGFR antibody [EP38Y] (ab52894). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

EGFRvIII expression in gliomas (DISCOVERY ULTRA)

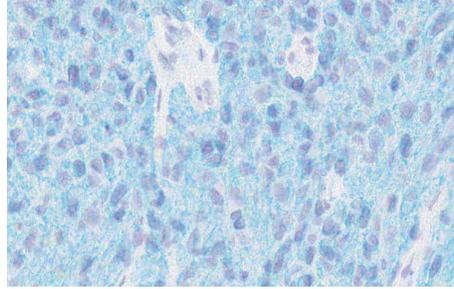
Below are the representative images of EGFRvIII and EGFR in glioblastoma, imaged using a duplex chromogenic assay.

Glioblastoma (high EGFRvIII frequency)

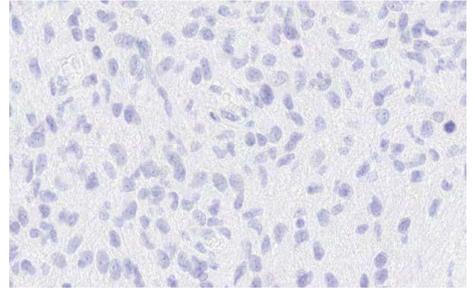
(a) EGFRvIII



(b) EGFR



(c) Neg Ctrl



(d) EGFRvIII / EGFR / Haematoxylin

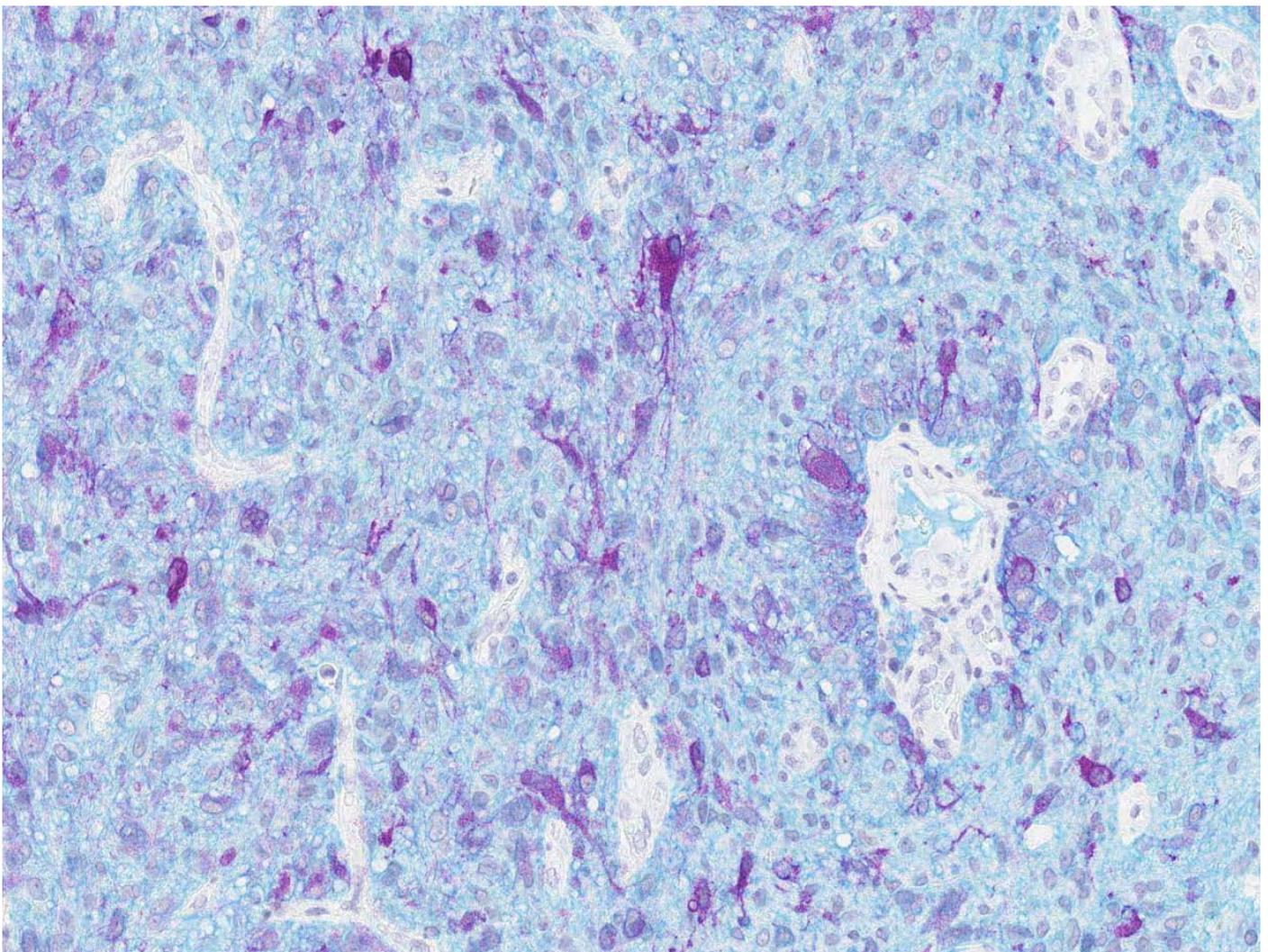


Figure 3. EGFRvIII specificity testing using a duplex co-localization assay.

IHC staining of sequential sections of human malignant glioblastoma tissues. The below table describes the staining conditions used in the duplex assay.

Figure	1 st Primary antibody (purple)	2 nd Primary antibody (teal)
3a	EGFRvIII	IHC diluent
3b	IHC diluent	EGFR
3c	IHC diluent	IHC diluent
3d	EGFRvIII	EGFR

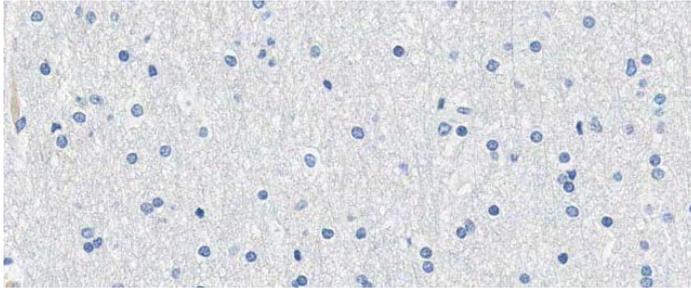
Hematoxylin nuclear counterstain in blue. Slides were scanned at 40x on Aperio® AT2 and imaged at 20x (a-c) and 10x (d) on Aperio® ImageScope.

EGFRvIII expression in human normal tissues (DISCOVERY ULTRA)

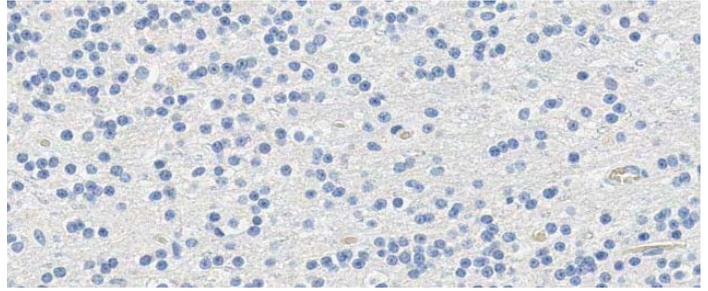
Below are the representative images of human multi-normal TMA where EGFRvIII expression was not detected in any of the 34 normal human tissues, as expected.

EGFRvIII

Cerebral cortex

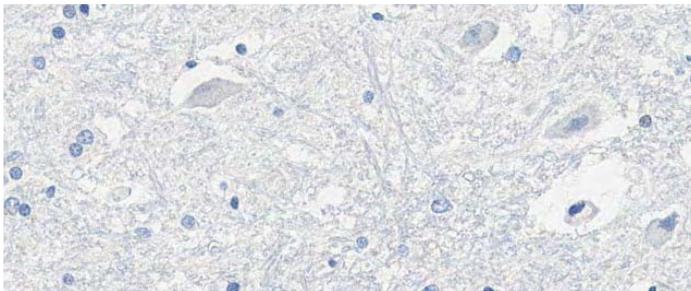


Cerebellum

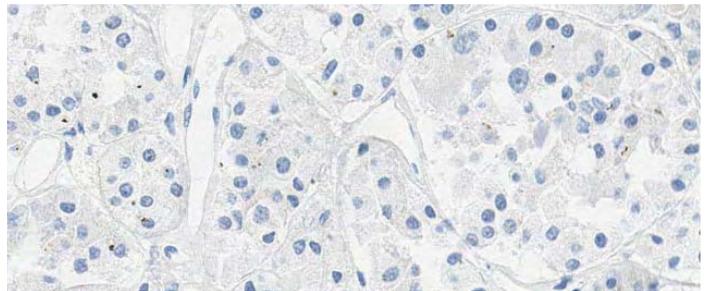


EGFRvIII

Spinal cord

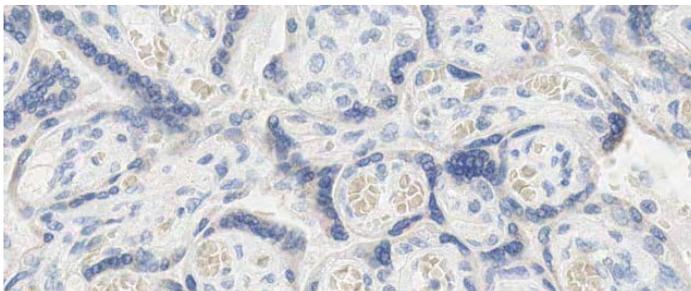


Pituitary gland



EGFRvIII

Placenta



Isotype control

Placenta

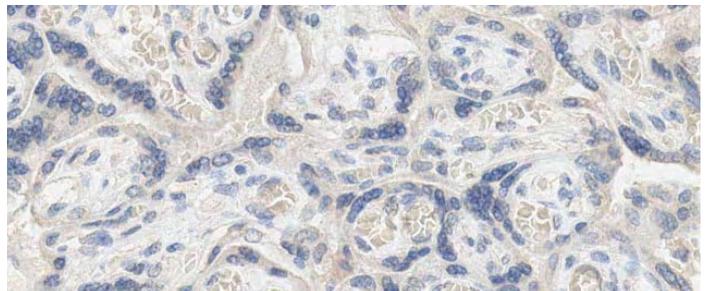


Figure 4. EGFRvIII expression in human normal tissues. IHC staining of normal human cerebrum, cerebellum, spinal cord, pituitary gland and placenta using anti-EGFRvIII recombinant antibody (ab313646) and anti-rabbit IgG isotype control (ab172730) showing the absence of staining. Hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

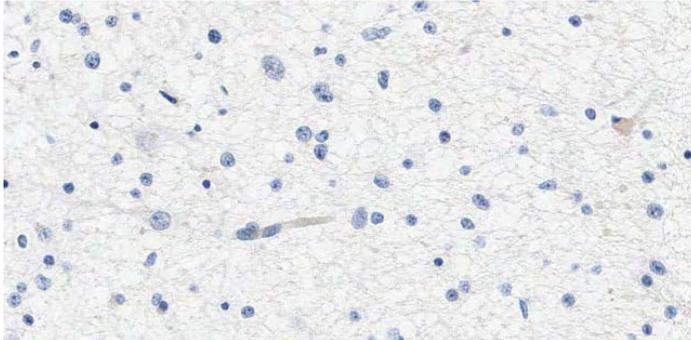
The multi-normal TMA consisted of other tissues (not shown) such as stomach, small intestine, colon, liver, lung, spleen, umbilical cord, parathyroid, thyroid, tonsil, bone marrow, fallopian tube, kidney, pancreas, skin, thymus, endometrium, urinary bladder, breast (2x), heart, ovary, prostate, testis, cervix, adrenal gland, lymph node, skeletal muscle and ureter. As expected, EGFRvIII expression was not detected.

EGFRvIII expression in human glioma TMA (DISCOVERY ULTRA)

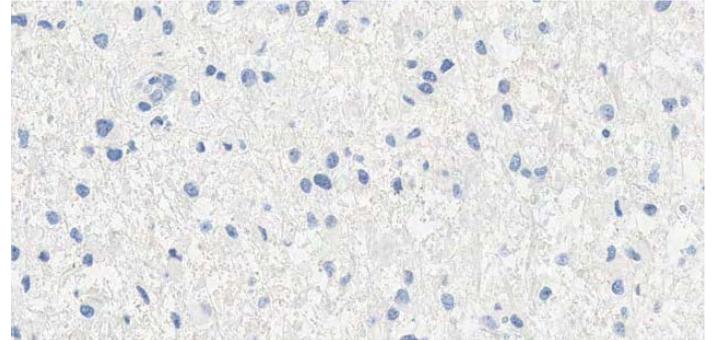
Below are the representative images of human glioma TMA consisting of various grades of gliomas (astrocytoma, oligodendroglioma, ependymoma).

EGFRvIII

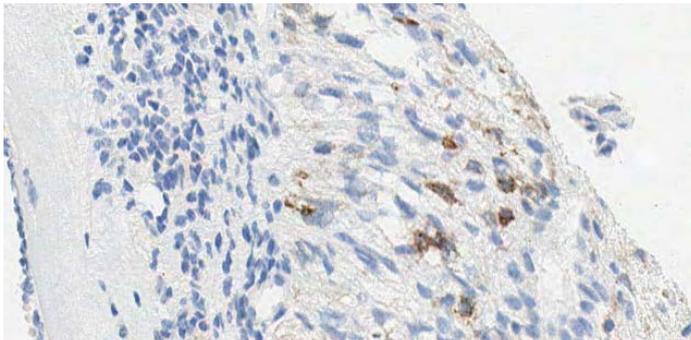
Pilocytic astrocytoma (Grade I)



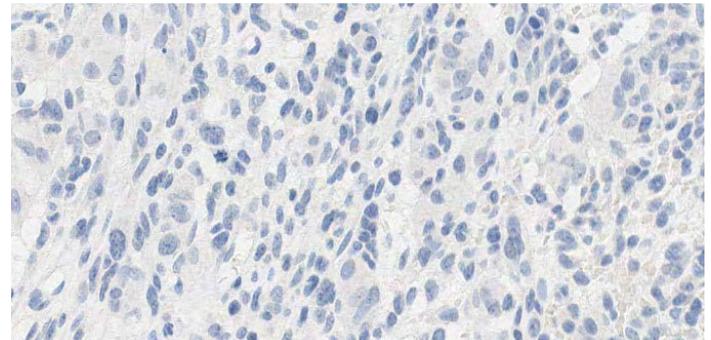
Diffuse astrocytoma (Grade II)



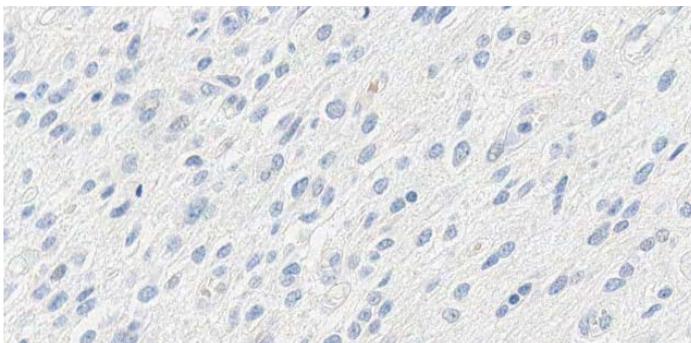
Anaplastic astrocytoma (grade III)



Glioblastoma



Oligodendroglioma



Glioblastoma / Isotype control

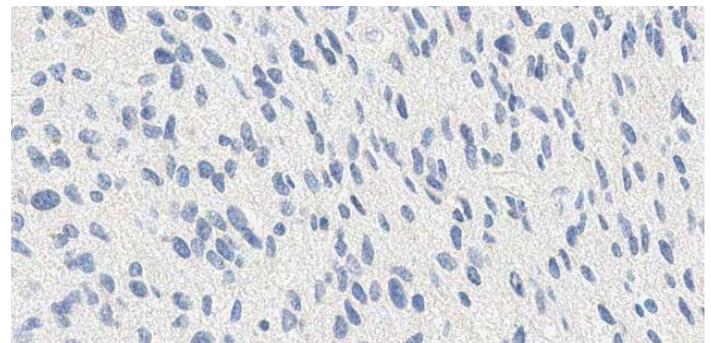


Figure 5. EGFRvIII expression in human glioma tissues. IHC staining of human glioma TMA using anti-EGFRvIII recombinant antibody (ab313646). EGFRvIII expression was detected in one individual case of grade III anaplastic astrocytoma. Absence of staining was observed in glioblastoma tissues when stained using anti-rabbit IgG isotype control antibody (ab172730). Positive staining in brown; Hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

References

1. Gan HK, Cvrljevic AN, Johns TG. The epidermal growth factor receptor variant III (EGFRvIII): where wild things are altered. *FEBS J*. 2013 Nov; **280**(21):5350-70. doi: 10.1111/febs.12393. Epub 2013 Jul 8. PMID: 23777544.
2. Gan HK, Kaye AH, Luwor RB. The EGFRvIII variant in glioblastoma multiforme. *J Clin Neurosci*. 2009 Jun; **16**(6):748-54. doi: 10.1016/j.jocn.2008.12.005. Epub 2009 Mar 25. PMID: 19324552.
3. Davis ME. Glioblastoma: Overview of Disease and Treatment. *Clin J Oncol Nurs*. 2016 Oct 1; **20**(5 Suppl):S2-8. doi: 10.1188/16.CJON.S1.2-8. PMID: 27668386; PMCID: PMC5123811.
4. An Z, Aksoy O, Zheng T, Fan QW, Weiss WA. Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. *Oncogene*. 2018 Mar; **37**(12):1561-1575. doi: 10.1038/s41388-017-0045-7. Epub 2018 Jan 11. PMID: 29321659; PMCID: PMC5860944.
5. Keller S, Schmidt MHH. EGFR and EGFRvIII Promote Angiogenesis and Cell Invasion in Glioblastoma: Combination Therapies for an Effective Treatment. *Int J Mol Sci*. 2017 Jun 18; **18**(6):1295. doi: 10.3390/ijms18061295. PMID: 28629170; PMCID: PMC5486116.
6. Chen R, Pan Y, Gutmann DH. The power of the few. *Genes Dev*. 2017 Jun 15; **31**(12):1177-1179. doi: 10.1101/gad.303453.117. PMID: 28765159; PMCID: PMC5558920.
7. Rutkowska A, Stoczyńska-Fidelus E, Janik K, Włodarczyk A, Rieske P. EGFRvIII: An Oncogene with Ambiguous Role. *J Oncol*. 2019 Dec **16**;2019:1092587. doi: 10.1155/2019/1092587. PMID: 32089685; PMCID: PMC7024087.

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