



## Alexa Fluor 488–conjugated anti-carcinoembryonic antigen monoclonal antibody

Alexa Fluor 488 anti-CEA MAb

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<b>Chemical name:</b>	Alexa Fluor 488–conjugated anti-carcinoembryonic antigen monoclonal antibody	
<b>Abbreviated name:</b>	AlexaFluor 488 anti-CEA MAb	
<b>Synonym:</b>		
<b>Agent Category:</b>	Monoclonal antibody	
<b>Target:</b>	Carcinoembryonic antigen (CEA)	
<b>Target Category:</b>	Antibody-ligand binding	
<b>Method of detection:</b>	Fluorescence imaging	
<b>Source of Signal/Contrast:</b>	Alexa Fluor 488	
<b>Activation:</b>	No	
<b>Studies:</b>	<ul style="list-style-type: none"> <li>• <i>In vitro</i></li> <li>• Rodents</li> </ul>	Click here for <a href="#">protein</a> and <a href="#">nucleotide</a> information regarding human CEA.

## Background

[PubMed]

Surgical resection is the most common treatment for colorectal and pancreatic cancers (1, 2). Therefore it is important to be able to detect the primary and metastatic lesions of these cancers for proper surgical resection and treatment. Although patients with colorectal cancers have more than one surgical option for treatment, the aggressive nature, and the usually late-stage detection, of pancreatic cancer often results in a negative outcome for patients (3). Also, chemotherapeutic treatments have a marginal effect on the survival of pancreatic cancer patients, which suggests that complete resection of the primary and metastatic lesions could possibly improve the chances of a positive treatment outcome (2, 4, 5). The carcinoembryonic antigen (CEA) has been shown to be a marker for several cancers originating from the endodermally derived epithelium of the digestive tract, including tissue from the embryonic gut, pancreas, and liver (6, 7). Because CEA is usually expressed at high levels in adenocarcinomas of the colon and the pancreatic ducts, it is used as a serum marker for the detection of

these cancers and to monitor patient response after treatment (8, 9). Kaushal et al. evaluated the use of a fluorophore-labeled anti-CEA monoclonal antibody (MAb) to detect colon and pancreatic cancer in nude mice bearing human xenograft tumors (10).

## Synthesis

[PubMed]

The anti-CEA MAb was purchased from commercial sources and conjugated with Alexa Fluor 488 dye according to the manufacturer's instructions (10). For the conjugation, the MAb was reconstituted in sodium bicarbonate and mixed with the dye as recommended by the manufacturer. The MAb-dye mixture was incubated for 1 h at room temperature and then overnight at 4°C. Unconjugated dye was removed with centrifugation on a column (type of column was not specified). A similar procedure was also used to conjugate the anti-CEA MAb with Oregon Green. A control IgG antibody was similarly labeled with the fluorophores. The fluorophore:MAb ratio of the labeled antibodies was reported to be 3–4:1 (10).

## In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Kaushal et al. investigated the expression of CEA with Alexa Fluor 488 anti-CEA MAb in several human cell lines of pancreatic or colon cancer origin (see Kaushal et al. for a list of all the cell lines (10)). Positive staining was indicated by fluorescence staining above background observed with the Alexa Fluor–conjugated IgG. Seven of the 10 pancreatic cell lines (70%) showed an expression of CEA. Among the colon cell lines, four of the six pancreatic cell lines (67%) were positive for the expression of CEA. A primary human colon cancer tissue, Colo4104, was also reported to express CEA. No blocking studies with the unconjugated anti-CEA MAb were reported.

## Animal Studies

### Rodents

[PubMed]

The fluorophore used for the *in vivo* studies was selected on the basis of comparing fluorescence imaging with the anti-CEA MAb conjugated to either the Oregon Green or the Alexa Fluor 488 dye (10). Animals ( $n = 3$  per fluorophore dose) bearing human pancreatic cancer cell ASPC-1 xenograft tumors were treated with various doses of either fluorophore-conjugated MAb and imaged 24 h later. The Alexa Fluor–conjugated MAb was reported to show the highest fluorescence intensity at the three lower doses with a marginal increase at the higher doses. The Oregon Green conjugated MAb had a lower fluorescence intensity compared to the Alexa Fluor–conjugated MAb at all doses.

Kaushal et al. investigated the expression of CEA in an *in vivo* athymic mouse model (10). The mice ( $n = 3$  animals per cell line) were respectively implanted (subcutaneously) with human ASPC-1, BxPC-3, CFPAC, Panc-1 and Capan-1 pancreatic cancer, LS174T colon cancer, and the primary Colo4104 cancer cell lines. The tumors were allowed to grow for 7–14 days; when the tumors were 1–2 mm in diameter, the animals were injected with the Alexa Fluor 488 anti-CEA MAb through the tail vein. All the tumors, including those from the primary colon cancer cell line, were reported to be positive for CEA. No competition binding studies were reported. A time course for the imaging of pancreatic tumors was also studied. Animals bearing human pancreatic cell tumors were injected with the fluorophore-conjugated MAb and imaged at various time periods from 30 min to 15 days ( $n = 2$  animals per time point) after treatment with the conjugated MAb. The

fluorescence signal was reported to steadily increase for up to 24 h after the conjugated-CEA MAb treatment, but it was observed to be minimal after 15 days.

CEA expression was also investigated in orthotopic tumors implanted in nude mice ( $n = 3$  animals per cell line) (10). Tumors were established with direct injection of BxPC-3 cells into the pancreas. Similarly, colorectal tumors were established in the animals with direct injection of the Colo4104 cells. The tumors were allowed to grow as described above, and the animals were treated with the conjugated-CEA MAb as described above. None of the tumors were easily visualized with standard bright light illumination, but with fluorescence imaging the lesions were very clear and the extent of invasion was observed to be much larger than apparent with standard illumination. The investigators reported that no fluorescence was observed when the animals were injected with the conjugated-IgG (control).

Alexa Fluor 488 anti-CEA MAb was also evaluated for the visualization of intra-abdominal metastases of pancreatic and colon cancers ( $n = 3$  animals per cell line) by implanting ASPC-1 (human pancreatic cancer origin) and Colo4104 (human colorectal cancer origin) cells into the peritoneal cavity of nude mice (10). The implants were allowed to grow for 1–2 weeks, and then the animals were injected with the conjugated-CEA MAb through the tail vein. Control animals received a fluorophore-conjugated IgG treatment. The animals were then imaged 24 h later with bright field and fluorescence illumination. Small peritoneal implants were reported to be visualized in the bowel and mesentery only with the conjugated-CEA MAb. No blocking studies with the unconjugated anti-CEA MAb were reported.

## Other Non-Primate Mammals

[PubMed]

No references are currently available.

## Non-Human Primates

[PubMed]

No references are currently available.

## Human Studies

[PubMed]

No references are currently available.

## Supplemental Information

[Disclaimers]

## NIH Support

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## References

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