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Antibodies for CFTR studies

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Abstract

For most expression studies focusing on the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, sensitive and specific antibodies (Abs) are critically needed. Several Abs have been produced commercially or by research laboratories for CFTR detection in both cell lines with heterologous or endogenous expression and native cells/tissues. Here, we review the applicability of most Abs currently in use in CF research for the biochemical and/or immunocytochemical detection of CFTR.

Keywords: Anti-CFTR; Antibodies; Immunotechniques; Immunocytochemistry; Western; Expression

1. Introduction

There are a variety of factors that influence the success of an immunochemical technique. According to some authors, these include (1) the avidity of the Ab for the antigen (Ag), (2) the concentration of the Ab and its specificity for the Ag, (3) possible alteration of the Ag epitope during the experimental procedure, (4) accessibility of the Ab to the Ag during the technique, and (5) type and quality of secondary reagents when used [1].

When CFTR is being detected, at least two additional factors should be considered, namely, the polarity status of cells under analysis and the levels of CFTR endogenous expression in cells or tissues, or copy number of transgene, in case of transfected cell lines [2].

Here, we review most Abs currently in use in CF research both polyclonal and monoclonal from commercial sources or produced by research laboratories, and summarize their applicability for the biochemical and/or immunocytochemical detection of CFTR, based on several comparative studies previously published [2–7]. Usage of anti-CFTR Abs described here includes detection in different types of samples, namely, (1) heterologous expression systems, (2) cell lines constitutively synthesizing the protein, and (3) native tissues.

Abbreviations: Ab, antibody; Ag, antigen; ELISA, enzyme-linked immunosorbent assay.

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Table 1
Review of anti-CFTR antibodies applicability

Antibody	Type[a]	Epitope	Source	Applicability[b]				Specificity	References[c]
				Cell lines		Native tissues/cells			
				WB	IP	ICC	IHC		
N-term-Birm[d]	PC	N-term (2–79)	D Cyr (Birmingham, AL, USA)	ND	T (+++)	ND	ND	H	[13]
MM13-4	MC (IgG1)	N-term (25–35)	Chemicon (Temecula, CA, USA)	T (+++)/E (+)	T (+++)/E (+)	N (+/–)	ND	H	[14]
MA1-935	MC (IgM)	1st EL (103-117)	Affinity Bioreagents (Golden, CO, USA)	ND	ND	ND	ND	H, M	[15,16]
PA1-935	PC (IgM)	1st EL (103-117)	Affinity Bioreagents	ND	ND	ND	ND	H	[15,16]
MATG 1031	MC (IgG1)	1st EL (107-117)	Transgène (Strasbourg, France)	ND	ND	ND	SG (–)	H	[17]
L12B4	MC (IgG2a)	Pre-NBD (386–412)	Chemicon	T (+++)/E (+)	T (+++)/E (–)	N (+/–)	ND	H, M, Ra	[14]
181	PC	Pre-NBD (415–427)	W Guggino (Baltimore, MD, USA)	Unsp	ND	ND	ND	H	[18,19]
MATG 1061	MC (IgG2a)	NBD1 (503–507/509–515)	Transgène	ND	ND	N (+++)	A (+++)/I (++)/SG (+)	H	[17,20]
NBD1-Birm	PC	NBD1-R	D Cyr	T (+++)/E (+)	T (+++)/E (+)	ND	ND	H	[2]
13-1	MC (IgG1)	R (590–830)	R&D Systems[e] (Abrington, UK)	ND	ND	N (+/–)	A (–)/I (++)	H	[21,22]
G449	PC	R (653–716)	H De Jonge	T (++)/E (+)	T (++)/E (+)	N (+++)[f]	I (++)	H	[23,24]
CC24-R	PC	R (693–716)	H De Jonge	T (+++)/E (++)	T (+)/E (–)	N (++)	A (–)/I/SG (++)	H	[23]
MATG 1104	MC (IgG1)	R (722–734)	Transgène	T (+++)/E (+/–)	T (–)/E (–)	N (++)[g]	A (++)/I (++)/SG (++)	H	[3,25]
169	PC	R (724–746)	W Guggino	ND	T (+++)/E (+)	N (++)	A (–)	H	[18,19]
M3A7	MC (IgG1)	NBD2-C-term (1197–1480)	Chemicon	T (+++)/E (+)	T (+++)/E (++)	N (+/–)	A (+/–)/I (+/–)/SG (++)	H, M, Ra	[3,14]
24-1	MC (IgG2a)	end NBD2-C-term (1377–1480)	R & D Systems[e]	T (+++)/E (++)	T (+++)/E (++)	N (+++)	A (++)/I (+/–)	H	[26–28]
GA-1	MC	end NBD2-C-term (1382–1480)	K Kirk (Birmingham, AL, USA)	_	T (+++)/E (+/–)	ND	ND	H	[29,30]
C1468	PC	C-term (1468–1480)	R Kopito (Stanford, CA, USA)	_	T (–)/E (ND)	ND	ND	H	[31,32]
Lis-1	PC	C-term (1468–1480)	MD Amaral	T (+++)	T (+++)	N (+++)	ND	H, M	[2,7]
R3195	PC	C-term (1468–1480)	C Marino (Memphis, TN, USA)	T (+++)/E (+)	T (+++)	ND	I (++)	H, M, Ra	[33–35]
MP-CT1	PC	C-term	R Dormer	T (+++)/E (++)	T (+++)/E (++)	N (+++)	A (++), I (++)	H, M	[36–38]

[a] Polyclonal (PC); monoclonal (MC).

[b] Based in Refs. [2–7]. For further details, please refer to original articles. Scale is from (+++) good detection, (++) reasonable detection, (+) poor detection, (–) no detection to unspecific (Unsp) or not determined (ND).

[c] Original reference, when applicable, in boldface.

[d] Abbreviations: C-terminus (C-term), extracellular loop (EL), Regulatory domain (R), nucleotide binding domain (NBD), N-terminus (N-term), Western blot (WB), immunoprecipitation (IP), immunocytochemistry (ICC), immunohistochemistry (IHC), endogenous (E), transfected (T), nasal (N), airways (A), intestine (I), sweat gland (SG), human (H), rat (Ra), mouse (M).

[e] These Abs were formerly available from Genzyme.

[f] H. Davidson and H. De Jonge, unpublished results.

[g] D. Penque and H. Davidson, unpublished results.

2. Materials and methods

2.1. Antibodies

All anti-CFTR Abs described here, as well as their original sources and/or references are presented in Table 1.

2.2. Affinity purification

The affinity purification of the antiserum usually improves the performance of an Ab. A good protocol can be found at the European Working Group on CFTR Expression website [8]. An alternative protocol is described elsewhere [2]. Briefly, antisera purification involves coupling of the epitope peptide to activated Sepharose beads, which are then used to fill up a column. The CFTR antiserum is then passed through this column and acid-eluted in 0.5-ml fractions, followed by a neutralization step. An additional step for desalting and concentration can be performed in columns designed for this purpose, with 10,000 MW cutoff limits [2]. The eluate fractions should then be assessed for efficiency in detecting the CFTR peptide epitope [e.g., on enzyme-linked immunosorbent assay (ELISA) plates] or CFTR directly (e.g., by Western blot). Affinity-purified Ab can be stored at 4 °C in the presence of sodium azide.

2.3. Immunodetection techniques

Consensus protocols for immunodetection of CFTR are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and [^{35}S]-labelling followed by immunoprecipitation of CFTR) are described by Farinha et al. [9]. Detection of CFTR protein by [^{32}P]-phosphorylation assays is described elsewhere [10]. The detection of CFTR by immunocytochemistry in native cells is described by Harris et al. [11] and by immunohistochemistry in tissues elsewhere [12].

3. Conclusion

CFTR protein is difficult to study and analysis based on detection of its presence is critically dependent on the use of robust Abs. We provide here a review of most anti-CFTR Abs available either from commercial or research sources that evidenced good results for the detection of wt- and F508del-CFTR by at least one immunochemical technique. The end-user researcher can thus save time and effort by choosing the Ab that best applies to the desired purpose.

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