



Legend for panels A through G

Symbols indicate temperature

- 22°C: circles
- 37°C: triangles
- 42°C: squares

Colors indicate antigens

- VLP: EEEV (green), VEEV (blue), WEEV (orange)
- E1 Protein: EEEV E1 (purple), CHIKV E1 (pink), MAYV E1 (red)
- E2 Protein: EEEV E2 (dark blue)

H EEEV-specific mAbs

Binding Group	EEEV mAb	Temperature-dependent binding EC ₅₀ (ng/mL)						VLP/Protein EC ₅₀ Ratio
		EEEV virus-like particles (VLPs)			EEEV E1 protein			
		22°C	37°C	42°C	22°C	37°C	42°C	22°C
EEEV VLP and E1 protein-reactive	EEEV-109	16	15	21	198	188	189	0.08
	EEEV-127	35	42	58	374	294	252	0.09
	EEEV-126	43	41	62	650	1,020	395	0.06
	EEEV-377	50	41	53	409	4,220	630	0.12
	EEEV-398	152	135	163	1,096	289	262	0.14
EEEV VLP > EEEV E1 protein	EEEV-400	44	35	55	>	>	>	NA
	EEEV-76	40	55	123	>	>	>	NA
	EEEV-104	105	102	115	>	>	>	NA
	EEEV-320	180	143	135	>	>	>	NA
EEEV E1 protein > EEEV VLP	EEEV-379	739	663	791	49	33	50	15.02
	EEEV-312	>	>	>	94	44	65	NA

I Cross-reactive mAbs

Cross reactivity	EEEV mAb	Temperature-dependent binding EC ₅₀ (ng/mL)								VLP/Protein EC ₅₀ Ratio
		EEEV virus-like particles (VLPs)				EEEV E1 protein				
		22°C	37°C	42°C	22°C	37°C	42°C	22°C		
EEEV-VEEV	EEEV-157	174	192	174	222	230	214	0.79		
	EEEV-179	49	55	40	2,686	1,100	1,651	0.09		
New World	EEEV-307	85	86	88	927	328	262	0.09		
	EEEV-354	94	107	84	410	235	348	0.23		
Broadly-reactive	EEEV-346	18	19	15	122	182	131	0.14		
	EEEV-387	118	69	130	232	626	351	0.51		
Pan-alphavirus	EEEV-138	23	25	26	194	249	292	0.12		
	EEEV-342	53	66	52	567	309	324	0.09		
	EEEV-368	83	58	81	317	265	237	0.26		
	EEEV-387	118	69	130	232	626	351	0.51		
	EEEV-387	118	69	130	232	626	351	0.51		

Legend for panels H and I

EC₅₀ (ng/mL)

- 0-100
- 101-500
- 501-5,000
- >5,001

VLP/Protein EC₅₀ Ratio

- 0-1.00
- 1.01-2.00
- 2.01-5.00
- >5.01